UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF TEXAS
SAN ANTONIO DIVISION

EKF-DIAGNOSTIC SALES, GmbH, a German Corporation,

Plaintiff-Counter-Defendant,

v.

HEMOCUE AB, a Swedish Corporation, and HEMOCUE, INC., a California Corporation,

Defendants-Counter-Claimants.

Civil Action No. SA04CA807(XR)
consolidated with SA03CA1080 (OG)

DEMAND FOR JURY TRIAL

EKF-DIAGNOSTIC SALES' FIRST AMENDED COMPLAINT FOR DECLARATORY JUDGMENT

Plaintiff EKF-Diagnostic Sales, GmbH (hereinafter "EKF-Sales") for its complaint against Defendants HemoCue AB and HemoCue, Inc. (hereinafter "HemoCue"), states as follows:

PARTIES AND NATURE OF ACTION

1. EKF-Sales is a German corporation, with its principal place of business at Ebendorfer Chaussee 3, Technologie Park Ostfalen, D–39179, Barleben, Magdeburg, Germany. EKF-Sales is involved in the distributorship/supply of special hemoglobin meters for human use and associated hemoglobin test cuvettes and accessories used with the meters.

2. On information and belief, HemoCue AB is a corporation organized under the laws of Sweden with its principal place of business at Box 1204SE-262 23 Angleholm, Sweden, and is purportedly the owner of U.S. Patent Number 5,674,457 ("the '457 Patent"), entitled

"Capillary Microcuvette," purportedly issued on October 7, 1997. A copy of the '457 Patent is provided herewith and attached as Exhibit A.

3. On information and belief, HemoCue, Inc., is a U.S. subsidiary of HemoCue AB and is a corporation organized under the laws of the state of California with its principal place of business at 40 Empire Drive, Lake Forest, California, 92630.

4. On information and belief, HemoCue, Inc. is the exclusive licensee of the '457 Patent.

5. This is an action for declaratory judgment that the '457 Patent is invalid, unenforceable due to inequitable conduct in procurement of the '457 Patent and not infringed by EKF-Sales, either directly or as an inducing or contributory infringer.

JURISDICTION AND VENUE

6. This Court has subject matter jurisdiction over this action pursuant to 28 U.S.C. § 2201 (declaratory judgment).

7. This Court has personal jurisdiction over the Defendants HemoCue because Defendants have sufficient contact with the state of Texas — in particular, this Judicial District — and maintenance of the suit in this Judicial District does not offend traditional notions of fair play and substantial justice. Venue is proper in this District pursuant to 28 U.S.C. § 1391. A substantial part of the Plaintiff's claims arise in this Judicial District.

FACTS COMMON TO THE COUNTS

8. EKF-Sales is a sales entity which sells, among other things, hemoglobin meters and associated test cuvettes.

9. Pursuant to an Agreement dated June 12, 2003, EKF-Sales entered into an exclusive sales and distributorship agreement with a company by the name of Stanbio Laboratory, L.P. ("Stanbio"), for purposes of selling and distributing hemoglobin meters and test cuvettes throughout the United States of America.

10. On information and belief, Stanbio is a limited partnership organized under the laws of the state of Texas, with its principal place of business at 1261 North Main Street, Boerne, Texas, 7806.

11. On information and belief, Defendant HemoCue AB manufactures hemoglobin meters and test cuvettes in Sweden and then sells them throughout the United States, including in Texas and this Judicial District, through its U.S. subsidiary Defendant HemoCue, Inc.

12. On information and belief, Defendants HemoCue AB and HemoCue, Inc. have attempted to assert rights under the '457 Patent against EKF-Diagnostic GmbH and against Plaintiff EKF-Sales' U.S. distributor Stanbio, purportedly based upon allegations that the test cuvettes purchased from EKF-Sales infringe the '457 Patent.

13. EKF-Diagnostic GmbH is a holding company having its place of business in Germany. EKF-Diagnostic GmbH does not manufacture or sell products in the United States. Furthermore, EKF-Diagnostic GmbH is not a party to any sales or distribution agreement with Stanbio.

14. On information and belief, Stanbio filed a complaint on October 29, 2003 in U.S. District Court, Western District of Texas, San Antonio Division (Civil Action No. SA 03 CA 1080 OG), seeking declaratory judgment that its offer for sale and sale of the cuvettes it purchases from Plaintiff EKF-Sales do not infringe the '457 Patent, that the '457 Patent is invalid, and that Stanbio's sale and offering for sale of such products is lawful.

15. On information and belief, on July 13, 2004, HemoCue filed a Motion to Dismiss Civil Action No. SA 03 CA 1080 (OG) for lack of subject matter jurisdiction alleging Stanbio's lack of a reasonable apprehension of being sued by HemoCue.

16. Pursuant to this Court's Order (Exhibit B) dated October 6, 2004, this Civil Action No. SA 04 CA 0807 (XR) has been consolidated with Stanbio's Civil Action No. SA 03 CA 1080 (OG).

17. On information and belief, on July 9, 2004, HemoCue filed a complaint in U.S. District Court, Southern District of California (Case No. 04 CV 1378 BEN (AJG)), alleging that

both Stanbio and EKF-Diagnostic GmbH (not EKF-Sales) infringe the '457 Patent. It is believed that HemoCue inadvertently named the wrong "EKF" entity. Regardless, neither EKF entities have any contact with the state of California.

18. As a result of HemoCue's complaint filed in California against the "wrong" EKF entity, albeit against the "correct" EKF ("EKF-Sales") distributor Stanbio, EKF-Sales has a reasonable fear and apprehension that HemoCue will commence an action for patent infringement of the '457 Patent against it in the United States. Accordingly, an actual existing and bona fide justiciable controversy therefore exists between EKF-Sales, HemoCue AB, and HemoCue, Inc. concerning the '457 patent, including its scope and its validity.

19. On information and belief, the '457 Patent relies upon the wrong science for supporting capillary force. As such, the '457 Patent fails to provide sufficient disclosure commensurate in scope to its claims to enable one of ordinary skill in the art to practice the alleged invention.

20. On information and belief, HemoCue knew that EKF-Sales' products rely on the teachings of at least one of HemoCue's earlier, not in force, patents.

HEMOCUE'S SALE OF TEST CUVETTES IN THE UNITED STATES

21. On information and belief, HemoCue AB introduced test cuvettes for hemoglobin in the United States market on or about 1986.

22. Since the introduction of the microcuvettes on the United States market, HemoCue's sale of microcuvettes in the United States alone exceeded over 60 million U.S. dollars in 2003, which is approximately 50% of its total global sales in 2003. This information can be obtained in HemoCue's website, www.hemocue.com/hemocueint/pdf/Busrep_03.pdf.

23. On information and belief, Jan Lilja and Sven Nilsson, listed inventors of the '457 Patent, co-founded HemoCue AB.

24. On information and belief, co-founders and listed inventors of the '457 Patent, Jan Lilja and Sven Nilsson, directly benefited and continue to benefit from HemoCue's sale of test cuvettes for hemoglobin in the United States market.

25. On information and belief, HemoCue's market share for the sale of test cuvettes for hemoglobin in the United States market was enhanced by U.S. Patent No. 4,088,448 (Exhibit C) (hereinafter "the '448 Patent") which was in force at the time when HemoCue entered the United States market.

26. HemoCue continues to dominate the United States market even after the '448 Patent (Exhibit C) expired in 1996.

27. This current market dominance is due in significant part to the '457 Patent being issued to HemoCue on October 7, 1996.

28. The '457 Patent is directed to improved test cuvettes for hemoglobin as was in the '448 Patent.

29. On information and belief and as will be alleged with particularity herein, HemoCue fraudulently procured the '457 Patent.

30. Once the '457 Patent issued, albeit fraudulently, HemoCue acquired the power to exclude competitors from selling hemoglobin test cuvettes in the United States which infringe the '457 Patent.

31. Accordingly, HemoCue contends the '457 Patent wrongfully excludes competitors, including EKF-Sales and Stanbio, from selling hemoglobin test cuvettes as described in the '457 Patent.

HEMOCUE'S FRAUDULENT PROCUREMENT OF THE '457 PATENT

32. On information and belief, co-founders of HemoCue AB, Jan Lilja and Sven Nilsson, began working on the concept of test cuvettes having at least one of the patented features of the '457 Patent at least as early as 1972.

33. On May 9, 1978, U.S. Patent No. 4,088,448 (Exhibit C), entitled "Apparatus for Sampling, Mixing the Sample with a Reagent and Making Particularly Optical Analyses" (hereinafter "the '448 Patent"), was issued to Jan Lilja and Sven Nilsson.

34. On March 31, 1987, U.S. Patent No. 4,654,197 (Exhibit D), entitled "Cuvette for Sampling and Analysis" (hereinafter "the '197 Patent"), was issued to Jan Lilja and Sven Nilsson.

35. On July 13, 1993, U.S. Des. Patent No. 337,388 (Exhibit E), entitled "Cuvette for an Optical Analysis of Liquids" (hereinafter "the '388 Patent"), was issued to Jan Lilja and Sven Nilsson.

36. On February 15, 1994, U.S. Patent No. 5,286,454 (Exhibit F), entitled "Cuvette" (hereinafter "the '454 Patent"), was issued to Jan Lilja and Sven Nilsson.

37. The inventors of the aforementioned prior art (Exhibits C-F), Jan Lilja and Sven Nilsson, are also listed inventors of the '457 Patent.

38. Inasmuch as the '448 Patent was disclosed as a relevant prior art by HemoCue during the prosecution of the '457 Patent, the '448 Patent (Exhibit C) is material to the patentability of the '457 Patent.

39. The '197 Patent (Exhibit D) discloses an integral capillary microcuvette comprising a body member having an outer peripheral edge, the body member being provided with a cavity that communicates with the outer peripheral edge of the body member, the cavity being defined by two opposing inner surfaces of the body member, a portion of the cavity defining a measuring zone within the body member.

40. On information and belief, the '197 Patent (Exhibit D) is material to the patentability of the '457 Patent.

41. The '388 Patent discloses an integral capillary microcuvette comprising a body member having a curved outer peripheral edge, the body member being provided with a cavity that communicates with the outer peripheral edge of the body member, the cavity being defined

by two opposing inner surfaces of the body member, a portion of the cavity defining a measuring zone within the body member.

42. On information and belief, the '388 Patent (Exhibit E) is material to the patentability of the '457 Patent.

43. The '454 Patent (Exhibit F) discloses an integral capillary microcuvette comprising a body member having an outer peripheral edge, the body member being provided with a cavity that communicates with the outer peripheral edge of the body member, the cavity being defined by two opposing inner surfaces of the body member, a portion of the cavity defining a measuring zone within the body member. Further, the '454 Patent (Exhibit F) states at column 3, lines 60-63 that:

> This "wick" 18 may be a conventional wick of any suitable material, but may also consist of special capillary slots in the cuvette walls or formations thereon.
>
> When using the cuvette according to FIGS. 1 and 2, the first cavity 12 is filled with a liquid sample which in the illustrated embodiment *is drawn into the cavity by capillary action* through the inlet 13. The liquid sample mixes with reagent or the like provided in the cavity 12, and the mixture can then be analysed, e.g., in a photometer. (Column 3, line 60 to column 4, line 2). (Emphasis added).

44. On information and belief, the '454 Patent (Exhibit F) is material to the patentability of the '457 Patent.

45. Notwithstanding the materiality of the aforementioned prior art (Exhibits C-F), HemoCue and/or at least one common inventor, Jan Lilja or Sven Nilsson, submitted only the '448 Patent (Exhibit C) to the Patent and Trademark Office during the prosecution of the '457 Patent.

46. On information and belief, HemoCue and/or at least one common inventor, Jan Lilja or Sven Nilsson, failed to disclose the '197 Patent (Exhibit D) to the Patent and Trademark Office during the prosecution of the '457 Patent.

47. On information and belief, HemoCue and/or at least the common inventor, Jan Lilja or Sven Nilsson, failed to disclose the '388 Patent (Exhibit E) to the Patent and Trademark Office during the prosecution of the '457 Patent.

48. On information and belief, HemoCue and/or at least one common inventor, Jan Lilja or Sven Nilsson, failed to disclose the '454 Patent (Exhibit F) to the Patent and Trademark Office during the prosecution of the '457 Patent.

49. On information and belief, HemoCue and/or at least one common inventor, Jan Lilja or Sven Nilsson, had a duty to disclose the '197 Patent (Exhibit D) to the Patent and Trademark Office.

50. On information and belief, HemoCue and/or at least one common inventor, Jan Lilja or Sven Nilsson, had a duty to disclose the '388 Patent (Exhibit E) to the Patent and Trademark Office.

51. On information and belief, HemoCue and/or at least one common inventor, Jan Lilja or Sven Nilsson, had a duty to disclose the '454 Patent (Exhibit F) to the Patent and Trademark Office.

52. Prior to introducing the test cuvettes for hemoglobin in the United States market, HemoCue provided the following documents to the Food and Drug Administration at least as early as 1984:

(a) A Microcuvette For The Determination of Total Hemoglobin In Blood (select portions provided in Exhibit G-1); and

(b) A Microcuvette For The Determination of Total Hemoglobin In Whole Blood (select portions provided in Exhibit G-2).

53. On information and belief, these documents (select portions provided in Exhibits G-1 and G-2) provided to the Food and Drug Administration reference patented features found in the '457 Patent.

54. On information and belief, these documents (select portions provided in Exhibits G-1 and G-2) provided to the Food and Drug Administration are material to the patentability of the '457 Patent.

55. On information and belief, HemoCue had a duty to disclose these documents (select portions provided in Exhibits G-1 and G-2) during the prosecution of the '457 Patent.

56. Nevertheless, these documents (select portions provided in Exhibits G-1 and G-2) were never provided to the Patent and Trademark Office during the prosecution of the '457 Patent.

57. On April 7, 1999, HemoCue filed U.S. Federal Trademark Registration Number 2629645 (Exhibit H) which shows a cuvette for use in hemoglobin testing.

58. This Trademark Registration Number 2629645 (Exhibit H) states that the logo shown therein was first used in commerce in 1982.

59. On information and belief, cuvettes having the design shown in this Trademark Registration Number 2629645 (Exhibit H) were sold prior to April 26, 1994. The logo discloses a cuvette having a space located between the cuvette's measuring zone and an end wall thereof, the space constituting an inner peripheral zone.

60. The test cuvettes for hemoglobin claimed in the '457 Patent have a space between its measuring zone and the end wall of the cuvette's inner peripheral zone.

61. On information and belief, the Trademark Registration Number 2629645 (Exhibit H) is material to the patentability of the '457 Patent.

62. On information and belief, HemoCue had duty to disclose the Trademark Registration Number 2629645 (Exhibit H) during the prosecution of the '457 Patent.

63. Nevertheless, the Trademark Registration Number 2629645 (Exhibit H) was never disclosed to the Patent and Trademark Office during the prosecution of the '457 Patent.

64. In view of the foregoing, HemoCue fraudulently procured the '457 Patent in the Patent and Trademark Office by failing to disclose material information (Exhibits D-H), in

violation of 37 C.F.R. §1.56, and are liable to EKF-Sales which has been injured thereby for any damages sustained in consequence thereof.

COUNT I

Declaratory Judgment For Non-Infringement

65. EKF-Sales repeats and realleges each and every allegation of paragraphs 1 through 64 as though fully set forth herein.

66. EKF-Sales seeks a declaratory judgment that the cuvettes it provides to Stanbio do not infringe the invention claimed in the '457 Patent.

67. EKF-Sales has not directly infringed, induced in the infringement of, nor has been a contributory infringer, of any of the claims of the '457 Patent.

COUNT II

Declaratory Judgment For Invalidity

68. EKF-Sales repeats and realleges each and every allegation of paragraphs 1 through 67 as though fully set forth herein.

69. EKF-Sales seeks a declaratory judgment that the '457 Patent is invalid.

70. HemoCue's 457 Patent is invalid and void, because the alleged invention was on sale more than one year prior to the date HemoCue filed its application for the '457 Patent in the United States, in violation of 35 U.S.C. § 102.

71. HemoCue's '457 Patent is invalid and void, because the alleged invention was described in the printed publications/patents more than one year prior to the date HemoCue filed its application for the '457 Patent in the United States, in violation of 35 U.S.C. § 102.

72. Alternatively, the claims of the '457 Patent are invalid as being obvious within the meaning of 35 U.S.C. § 103 over the printed publications/patents described herein.

COUNT III

Declaratory Judgment For Lawful Sale And Offering To Sell

73. EKF-Sales repeats and realleges each and every allegation of paragraphs 1 through 72 as though fully set forth herein.

74. EKF-Sales seeks a declaratory judgment that its sale and offering to sell its products is lawful.

COUNT IV

Declaratory Judgment for Exceptional Case Resulting From Inequitable Conduct and Fraud on the Patent and Trademark Office

75. EKF-Sales repeats and realleges each and every allegation of paragraph 1 through 74 as though fully set forth herein.

76. On information and belief, HemoCue caused the application which matured into the '457 Patent to be filed with full knowledge that the subject matter of the application had previously been invented by at least two inventors of the '457 Patent, Jan Lilja and Sven Nilsson.

77. On information and belief, at least one inventor, Jan Lilja or Sven Nilsson, was associated with the filing or prosecution of the '457 Patent.

78. On information and belief, the claimed subject matter of the '457 Patent was described, in full or in part, in printed publications more than one year prior to the April 26, 1995 filing date thereof. Specifically, the claimed subject matter of the '457 Patent was described in full or in part within: "A Microcuvette for The Determination of Total Hemoglobin in Blood" (select portions provided in Exhibit G-1) published at least as early as in 1984; "A Microcuvette for The Determination of Total Hemoglobin in Whole Blood" (select portions provided in Exhibit G-2) published at least as early as in 1984; and U.S. Trademark Registration Number 2629645 (Exhibit H) which identifies a use since 1982 of cuvettes having patented features.

79. On information and belief, during prosecution of the '457 Patent and in breach of its duty, HemoCue failed to advise the Patent Examiner of the aforementioned documents (Exhibits G-H) listed in paragraph 75.

80. On information and belief, a reasonable patent examiner would find the document "A Microcuvette for The Determination of Total Hemoglobin in Blood" (select portions provided in Exhibit G-1) to be material to the patentability of the '457 patent.

81. On information and belief, HemoCue intentionally withheld the document "A Microcuvette for the Determination of Total Hemoglobin in Blood" (select portions provided in Exhibit G-1) during the prosecution of the '457 Patent.

82. On information and belief, HemoCue's failure to disclose the document "A Microcuvette for the Determination of Total Hemoglobin in Blood" (select portions provided in Exhibit G-1) during the prosecution of the '457 patent reveals a calculated recklessness about the truth.

83. On information and belief, a reasonable patent examiner would find the document "A Microcuvette for The Determination of Total Hemoglobin in Whole Blood" (select portions provided in Exhibit G-2) to be material to the patentability of the '457 patent.

84. On information and belief, HemoCue intentionally withheld the document "A Microcuvette for the Determination of Total Hemoglobin in Whole Blood" (select portions provided in Exhibit G-2) during the prosecution of the '457 Patent.

85. On information and belief, HemoCue's failure to disclose the document "A Microcuvette for the Determination of Total Hemoglobin in Whole Blood" (select portions provided in Exhibit G-2) during the prosecution of the '457 patent reveals a calculated recklessness about the truth.

86. On information and belief, a reasonable patent examiner would find the document U.S. Trademark Registration Number 2629645 (Exhibit H) to be material to the patentability of the '457 patent.

87. On information and belief, HemoCue intentionally withheld the document U.S. Trademark Registration Number 2629645 (Exhibit H) during the prosecution of the '457 Patent.

88. On information and belief, HemoCue's failure to disclose the document U.S. Trademark Registration Number 2629645 (Exhibit H) during the prosecution of the '457 patent reveals a calculated recklessness about the truth.

89. Nevertheless, HemoCue failed to disclose the aforementioned documents (Exhibits G-H) to the Patent and Trademark Office during the prosecution of the '457 Patent.

90. Furthermore, HemoCue failed to disclose the existence of the '197 Patent (Exhibit D) to the Patent Office during the prosecution of the '457 Patent.

91. HemoCue also failed to disclose the existence of the '388 Patent (Exhibit E) to the Patent Office during prosecution of the '457 Patent.

92. HemoCue also failed to disclose the existence of the '454 Patent (Exhibit F) to the Patent Office during the prosecution of the '457 Patent.

93. Jan Lilja is a named inventor on the '197 Patent.

94. On information and belief, Jan Lilja is a co-founder of HemoCue AB.

95. On information and belief, Jan Lilja has financially benefited either directly or indirectly as a result of the issuance of the '457 Patent.

96. Seven Nilsson is a named inventor on the '197 Patent.

97. On information and belief, Sven Nilsson is a co-founder of HemoCue AB.

98. On information and belief, Sven Nilsson has financially benefited either directly or indirectly as a result of the issuance of the '457 Patent.

99. Jan Lilja and Sven Nilsson are named inventors on the '197 Patent.

100. The '197 Patent discloses features claimed by the '457 Patent.

101. The '197 Patent, by itself or in combination with other references cited during the prosecution of the '457 Patent, would invalidate at least one claim of the '457 Patent under 35 U.S.C. §§ 102 or 103.

102. On information and belief, a reasonable patent examiner would find the '197 Patent to be material to the patentability of the '457 patent.

103. On information and belief, Jan Lilja intentionally withheld the '197 Patent during the prosecution of the '457.

104. On information and belief, Jan Lilja's failure to disclose the '197 Patent during the prosecution of the '457 Patent reveals a calculated recklessness about the truth.

105. On information and belief, Jan Lilja knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '197 Patent during the prosecution of the '457 patent.

106. On information and belief, for financial benefit, Jan Lilja knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '197 Patent during the prosecution of the '457 patent.

107. On information and belief, Sven Nilsson knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '197 Patent during the prosecution of the '457 patent.

108. On information and belief, for financial benefit, Sven Nilsson knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '197 Patent during the prosecution of the '457 patent.

109. On information and belief, Sven Nilsson intentionally withheld the '197 Patent during the prosecution of the '457.

110. On information and belief, Sven Nilsson's failure to disclose the '197 Patent during the prosecution of the '457 Patent reveals a calculated recklessness about the truth.

111. Jan Lilja and Sven Nilsson are named inventors on the '388 Patent.

112. The '388 Patent discloses features claimed by the '388 Patent.

113. The '388 Patent, by itself or in combination with other references cited during the prosecution of the '457 Patent, would invalidate at least one claim of the '457 Patent under 35 U.S.C. §§ 102 or 103.

114. On information and belief, a reasonable patent examiner would find the '388 Patent to be material to the patentability of the '457 patent.

115. On information and belief, Jan Lilja intentionally withheld the '388 Patent during the prosecution of the '457.

116. On information and belief, Jan Lilja's failure to disclose the '388 Patent during the prosecution of the '457 Patent reveals a calculated recklessness about the truth.

117. On information and belief, Jan Lilja knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '388 Patent during the prosecution of the '457 patent.

118. On information and belief, for financial benefit, Jan Lilja knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '388 Patent during the prosecution of the '457 patent.

119. On information and belief, Sven Nilsson knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '388 Patent during the prosecution of the '457 patent.

120. On information and belief, for financial benefit, Sven Nilsson knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '388 Patent during the prosecution of the '457 patent.

121. On information and belief, Sven Nilsson intentionally withheld the '388 Patent during the prosecution of the '457.

122. On information and belief, Sven Nilsson's failure to disclose the '388 Patent during the prosecution of the '457 Patent reveals a calculated recklessness about the truth.

123. Jan Lilja and Sven Nilsson are named inventors on the '454 Patent.

124. The '454 Patent discloses features claimed by the '457 Patent.

125. The '454 Patent, by itself or in combination with other references cited during the prosecution of the '457 Patent, would invalidate at least one claim of the '457 Patent under 35 U.S.C. §§ 102 or 103.

126. On information and belief, a reasonable patent examiner would find the '454 Patent to be material to the patentability of the '457 patent.

127. On information and belief, Jan Lilja intentionally withheld the '454 Patent during the prosecution of the '457.

128. On information and belief, Jan Lilja's failure to disclose the '454 Patent during the prosecution of the '457 Patent reveals a calculated recklessness about the truth.

129. On information and belief, Jan Lilja knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '454 Patent during the prosecution of the '457 patent.

130. On information and belief, for financial benefit, Jan Lilja knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '454 Patent during the prosecution of the '457 patent.

131. On information and belief, Sven Nilsson knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '454 Patent during the prosecution of the '457 patent.

132. On information and belief, for financial benefit, Sven Nilsson knowingly breached the duty under 37 CFR §1.56 to provide the Patent and Trademark Office with the '454 Patent during the prosecution of the '457 patent.

133. On information and belief, Sven Nilsson intentionally withheld the '454 Patent during the prosecution of the '457.

134. On information and belief, Sven Nilsson's failure to disclose the '454 Patent during the prosecution of the '457 Patent reveals a calculated recklessness about the truth.

136. The '457 Patent is, therefore, unenforceable due to HemoCue's and/or at least one common inventor's, Jan Lilja or Sven Nilsson, inequitable conduct and fraud upon the Patent and Trademark Office during the prosecution and due to the issuance of the '457 Patent in violation of 35 U.S.C. §§ 102 and 103.

137. Despite HemoCue's full knowledge of the unenforceability of the '457 Patent due to inequitable conduct, HemoCue has sought to enforce the '457 Patent in the United States

federal court against EKF and Stanbio. Such conduct renders this case exceptional under 35 U.S.C § 285, and thereby entitles EKF to recover its attorneys' fees necessitated by this lawsuit.

PRAYER FOR RELIEF

WHEREFORE, Plaintiff EKF-Sales requests judgment against Defendants HemoCue AB and HemoCue, Inc. for the following:

A. Judgment that EKF-Sales' cuvettes do not infringe U.S. Patent No. 5,674,457;

B. Judgment that U.S. Patent No. 5,674,457 is invalid;

C. Judgment that EKF-Sales' sale and offering to sell its cuvettes is lawful;

D. Judgment that HemoCue fraudulently procured U.S. Patent No. 5,674,457.

E. Judgment that this case is exceptional with the meaning of 35 U.S.C. § 285 and award EKF-Sales its attorneys' fees, costs, and expenses in this action; and

F. Provide any further relief as this Court may deem equitable and proper.

JURY DEMAND

Plaintiff EKF-Sales demands trial by jury of all issues triable to a jury.

Dated: May 02, 2005

Respectfully submitted,

FACTOR & LAKE, LTD

By: _____

Micheal D. Lake, Esq.
Edward L. Bishop, Esq.
1327 W. Washington Blvd. Suite 5G/H
Chicago, IL 60607
(312) 226-1818
(312) 226-1919 (facsimile)

Attorneys for Plaintiff,
EKF-Diagnostic Sales, GmbH

By mdl
by permission

CERTIFICATE OF SERVICE

I hereby certify that on this, 2 day of May 2005, a true and correct copy of the foregoing **EKF-DIAGNOSTIC SALES' FIRST AMENDED COMPLAINT FOR DECLARATORY JUDGMENT** was served via certified mail, return receipt requested upon the following:

Counsel for Stanbio Laboratory, L.P.
Mark H. Miller, Esq.
Jackson Walker, L.L.P.
112 East Pecan, Suite 2100
San Antonio, TX 78205

Counsel for Hemocue Inc. and Hemocue AB
Dean V. Fleming, Esq.
FULLBRIGHT & JAWORSKI
300 Convent, Suite 2200
San Antonio, TX 78205

Counsel for Hemocue Inc. and Hemocue AB
Paul Krieger, Esq.
Edward D. Steakley, Esq.
FULLBRIGHT & JAWORSKI, LLP
1301 McKinney, Suite 5100
Houston, TX 77010-3095

Counsel for EKF-Diagnostic Sale, GmbH
John K. Schwartz, Esq.
LOCKE LIDDELL & SAPP, L.L.P.
100 Congress Avenue, Suite 300
Austin, TX 78701-4042

Edward L. Bishop

by Mark Miller

by permission

US005674457A

United States Patent [19]

Williamsson et al.

[11] Patent Number: 5,674,457

[45] Date of Patent: Oct. 7, 1997

[54] **CAPILLARY MICROCUVETTE**

[75] Inventors: **Anders Williamsson**, Helsingborg; **Stefan Wahlqvist**, Lomma; **Sven-Erik Nilsson**; **Jan Lilja**, both of Helsingborg; **Lars Jansson**, Ängelholm; **Bertil Nilsson**, Bjärred, all of Sweden

[73] Assignee: **Hemocue AB**, Ängelholm, Sweden

[21] Appl. No.: **429,494**

[22] Filed: **Apr. 26, 1995**

[51] **Int. Cl.**[6] **B01L 3/00**

[52] **U.S. Cl.** **422/102**; 422/104; 422/99; 356/246

[58] **Field of Search** 422/99, 100, 102, 422/104; 356/246, 440

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,015,949	10/1935	Maw	356/246
2,056,791	10/1936	Logan	356/246
2,059,374	11/1936	Logan et al.	356/246
2,062,587	12/1936	Logan et al.	356/246
2,062,588	12/1936	Logan et al.	356/70
2,069,374	2/1937	Lagomarsino	62/298
3,363,503	1/1968	Shifrin	356/246
3,501,242	3/1970	De Mey, II et al.	356/246
3,565,537	2/1971	Fielding	356/246
3,698,822	10/1972	Polanyi	356/246
3,705,000	12/1972	Guerra	356/246
3,814,522	6/1974	Clark et al.	356/197
4,088,448	5/1978	Lilja et al.	422/102
4,088,488	5/1978	Chang et al.	96/29 D
4,405,235	9/1983	Rossiter	356/246
4,596,695	6/1986	Cottingham	422/58
4,746,215	5/1988	Gross	356/339
4,756,884	7/1988	Hillman et al.	422/73
4,761,381	8/1988	Blatt et al.	436/165
4,865,812	9/1989	Kuntz et al.	422/99
4,957,582	9/1990	Columbus	156/332
4,981,654	1/1991	Kuntz et al.	422/102
5,030,421	7/1991	Muller	422/102
5,147,607	9/1992	Mochida	422/57
5,260,032	11/1993	Muller	422/102

FOREIGN PATENT DOCUMENTS

0 287 883	10/1988	European Pat. Off. .
2-17426	1/1990	Japan .

OTHER PUBLICATIONS

"An Azide–Methemoglobin Method for Hemoglobin Determination in Blood", Guilio Vanzetti, J. Lab. & Clin. Med., vol. 67, No. 1, pp. 116–126, Jan. 1966.

Primary Examiner—Harold Pyon

Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis, L.L.P.

[57] **ABSTRACT**

The present invention is related to an integral capillary microcuvette comprising a body member and a cavity including a measuring zone within the body member. The cavity is defined by two opposite, substantially parallel inner surfaces of the body member and includes an outer peripheral edge comprising a sample inlet and an inner peripheral zone having a channel of higher capillary force than the measuring zone. The channel extends around the entire inner peripheral zone with ends of the channel communicating with the atmosphere at the exterior of the microcuvette.

7 Claims, 2 Drawing Sheets

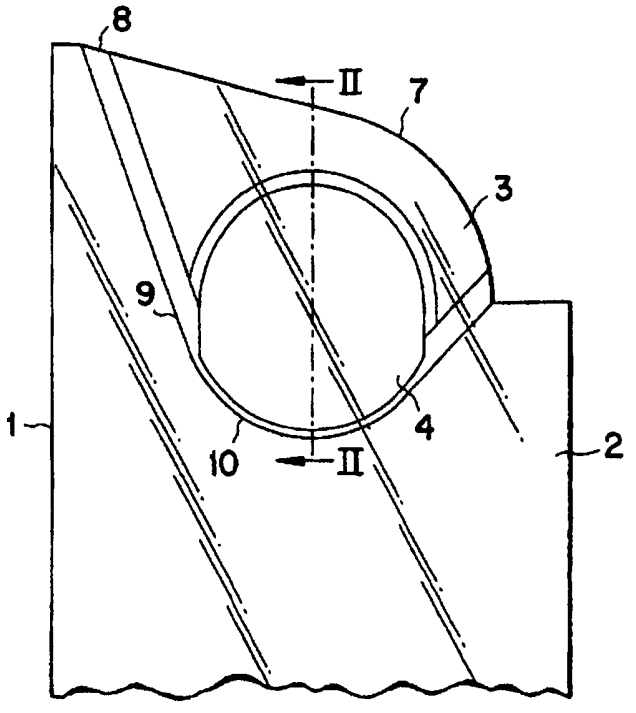
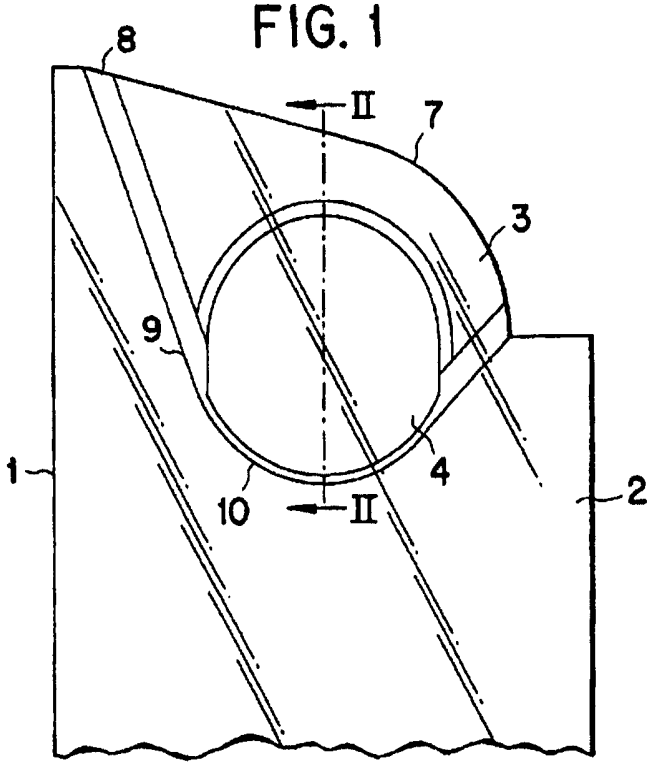


FIG. 1



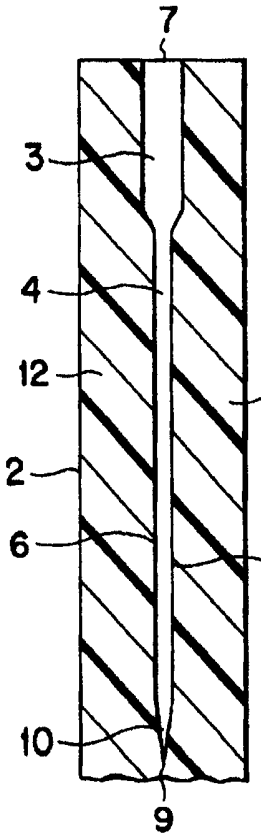
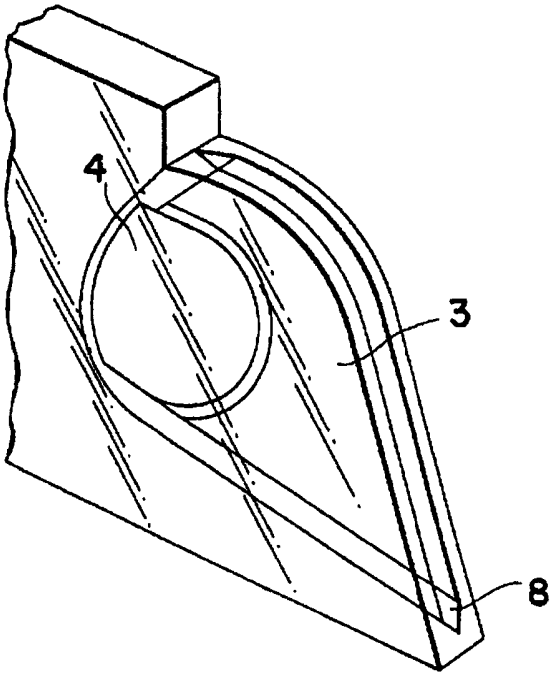


FIG. 2

FIG. 3



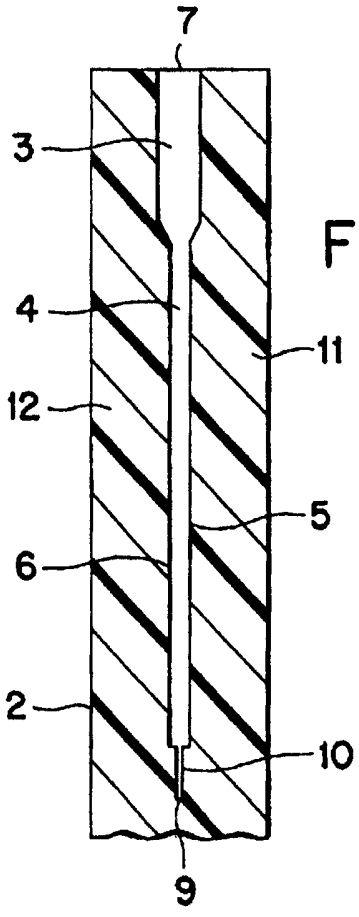


FIG. 4

CAPILLARY MICROCUVETTE

BACKGROUND OF THE INVENTION

The present invention concerns a capillary microcuvette. More specifically the invention concerns a disposable integral capillary microcuvette having improved flow for essentially simultaneously sampling a fluid and analyzing of the sample.

A cuvette for sampling a fluid, mixing the sample with a reagent and directly making optical analysis of the sample mixed with the reagent is previously known from U.S. Pat. No. 4,088,448. This cuvette comprises a body member including two planar surfaces defining an optical path and placed at a predetermined distance from one another to determine the optical path length and to define a cavity which includes a measuring zone therein, having an inlet for communicating said cavity with the exterior of the body member. The cavity has a predetermined fixed volume, and the predetermined distance permits the sample to enter the cavity by capillary force. Furthermore, a reagent is coated on the cavity surface, which mixes with the sample and allows the sample to be measured by optical analysis.

This known cuvette has several advantages when compared with the conventionally used devices. It permits sampling of a liquid, mixing and chemically reacting it with a suitable reagent; e.g. for colour development, in the same vessel as the one used for the subsequent measurement. The cuvette disclosed in U.S. Pat. No. 4,088,448 thus simplifies the sampling procedure, reduces the number of devices needed and in most cases, depending on the type of analysis, considerably improves the accuracy of the analysis by making the analyzing procedure independent of the operation of the device.

However, it has been discovered that the microcuvette described in U.S. Pat. No. 4,088,448 may develop air bubbles that can interfere with the optical analysis. Air bubbles generally form in the cavity of the cuvettes because of unsatisfactory sample flow in the cuvette cavity. This is especially detrimental for hemoglobin measurements because of the strong absorption of the hemoglobin. In particular, in a photometric determination, the presence of a large air bubble in the light path traversing the measuring zone will result in an overall measured hemoglobin value below the actual level because the photometer will read the bubble as a contribution of extremely low hemoglobin. Quality control is routinely carried out to discard those cuvettes which include air bubbles, thereby eliminating the risk that air bubbles will be present in the measuring zone when the cuvettes are used in a clinical procedure. A considerable number of cuvettes do not pass the quality control and have to be discarded, thereby increasing the overall cost of the cuvettes.

OBJECT OF THE INVENTION

One object of the present invention is to provide an improved cuvette which eliminates the risk of failure caused by the presence of air bubbles in the measuring zone.

SUMMARY OF THE INVENTION

The above objects and others are accomplished by providing a disposable, integral capillary microcuvette for essentially simultaneous sampling a fluid and analyzing the sample. In connection with the present invention the term "integral" means that the cuvette is made or manufactured in one, integral, piece. The microcuvette comprises a body member and a cavity including a measuring zone within the body member. The cavity is defined by two opposite, substantially parallel inner surfaces of the body member and includes an outer peripheral edge comprising a sample inlet and an inner peripheral zone having a channel of higher capillary force than the measuring zone. The channel extends around the entire inner peripheral zone with ends of the channel communicating with the exterior of the microcuvette.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a plan view of the microcuvette according to one embodiment of the present invention.

FIG. 2 is a cross sectional view of a microcuvette according to the present invention, taken along line II—II of FIG. 1.

FIG. 3 is a perspective view of the microcuvette according to the invention.

FIG. 4 is a cross-sectional view of a microcuvette according to another embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

FIG. 1 is a plan view of a microcuvette generally designated by reference numeral 1, according to one embodiment of the present invention. The microcuvette 1, comprises a body member 2, comprised of two substantially planar sheets of material 11, 12, and includes a cavity 3, defined by two inner surfaces 5, 6, of the body member 2. A measuring zone 4 is arranged within the cavity 3. The distance between the surfaces 5, 6, defining the measuring zone 4, is a critical parameter in providing the proper optical path length for the desired measurement. In a preferred embodiment of measuring hemoglobin, the distance should be between 0.05 and 0.15 mm. The distance between the inner surfaces of the rest of the cavity 3 is preferably in the order of 0.3–2 mm, i.e. clearly longer than the distance between the inner surfaces 5, 6 of the measuring zone. An outer peripheral edge 7, includes a sample inlet 8, comprised of the opening between the two sheets 11, 12, making up the body member 2. An inner peripheral zone 9, includes a channel 10, which has a higher capillary force than the measuring zone 4. The channel 10, which can have any shape, extends along the entire inner peripheral zone 9, and communicates with the atmosphere at both ends of the channel 10. The channel 10, preferably has a width between 10 micron and 2 mm.

When a sample liquid is drawn into the cuvette through the inlet 8, the channel 10 is filled along its entire length due to its high capillary action. After filling of the channel the sample liquid propagates into the rest of the cavity 3 in a flow pattern which prevents air bubbles to be captured in the measuring zone 4.

The provision of the channel having a higher capillary force than the measuring zone thus improves hydrodynamic flow within the cuvette cavity and prevents air bubbles to be trapped in the measuring zone. The channel may have any appropriate shape or form as long as the capillary force of the channel is higher than the capillary force of the measuring zone. This is accomplished by providing a channel having a depth which is less than that of the measuring zone. In particular, the channel may be defined by an inner wall of the inner peripheral zone and by the two opposite, substantially planar, surfaces of the body member whereby the distance between the planar surfaces of the channel is

shorter than the distance between the inner surfaces of the measuring zone as shown in FIG. 3.

In an alternative embodiment of the present invention, the distance between the two opposite substantially planar surfaces of the body member continuously increases in a direction extending away from the inner end wall of the inner peripheral zone. In this case the channel is shaped as a wedge, the bottom of which opens towards the measuring zone.

The cuvettes according to the present invention may be formed from any suitable material which allows the formation of the channel and measuring zone to the necessary tight tolerance levels. Preferably, the cuvettes according to the present invention are made of glass or a polymeric material.

Cuvettes according to the present invention were compared with cuvettes according to U.S. Pat. No. 4,088,448 as follows:

A reagent of

40 g sodium desoxycholate

18 g sodium azid and

20 g sodium nitrite

per liter solvent was prepared.

100 cuvettes according to U.S. Pat. No. 4,088,448 available from HemoCue AB, Sweden, and 100 cuvettes according to the present invention were filled with the above reagent, air dried and examined optically for uniform drying pattern. The cuvettes were then filled with whole blood, EDTA and an anticoagulating agent. A hemoglobin measurement was then carried out according to a modified azidmethemoglobin method according to Vanzetti described in J. Lab. Clin. Med. 67, 116–26 (1966) wherein the measurement is made at 570 and 880 nm respectively. The number of cuvettes which exhibited air bubbles was recorded.

Type of Cuvette	Number with air bubble
U.S. Pat. No. 4,088,448	25
The invention	0

As is apparent from the above, the cuvettes according to the present invention are very advantageous in eliminating the risks associated with the occurrence of air bubbles within the measuring zone. By providing the cuvette according to the present invention with a channel having higher capillary force than that of the measuring zone, air bubbles were entirely eliminated. This not only reduced the costs associated with discarded cuvettes but also greatly reduces the risk of improper readings which occur because of air bubbles.

The present invention has been described above with respect to the measurement of hemoglobin. However, the present invention is equally applicable to the measurement of other blood chemistry values, such as glucose, blood urea nitrogen, albumin, bilirubin, and total protein, etc. Furthermore, the present invention is applicable to numerous other analytical measurements and tests outside the blood chemistry field.

The foregoing has been a description of certain preferred embodiments of the present invention, but it is not intended to limit the invention in any way. Rather, many modifications, variations, and changes in details may be made within the scope of the present invention.

What is claimed is:

1. An integral capillary microcuvette comprising a body member having an outer peripheral edge, the body member being provided with a cavity that communicates with the outer peripheral edge of the body member, the cavity being defined by two opposing inner surfaces of the body member, a portion of the cavity defining a measuring zone within the body member, the cavity having an inner peripheral zone at which is located a channel, the channel extending along the entire inner peripheral zone of the cavity, the channel being sized relative to the measuring zone such that the channel has a higher capillary force than the measuring zone to prevent air bubbles from becoming trapped in the measuring zone, the outer peripheral edge of the body member being provided with a sample inlet through which a sample is drawn into the body member, the sample inlet being in communication with the channel and the channel being in communication with the measuring zone.

2. A microcuvette according to claim **1**, wherein said cavity has a predetermined volume.

3. A microcuvette according to claim **1**, wherein said cavity includes a dry reagent in a predetermined amount.

4. A microcuvette according to claim **1**, wherein the distance between the inner surfaces of the body member at said measuring zone does not exceed 0.15 mm.

5. A microcuvette according to claim **1**, wherein said channel is defined by an inner end wall at said inner peripheral zone and two substantially planar portions of the inner surfaces of said body member.

6. A microcuvette according to claim **5**, wherein said two substantially planar portions are parallel and the distance between the two substantially planar portions is less than the distance between portions of the inner surfaces of the body member at said measuring zone.

7. A microcuvette according to claim **5**, wherein the distance between the two substantially planar surfaces of said body member increases in a direction extending away from said inner end wall of said inner peripheral zone.

* * * * *

IN THE UNITED STATES DISTRICT COURT
FOR THE WESTERN DISTRICT OF TEXAS
SAN ANTONIO DIVISION

EKF-DIAGNOSTIC SALES, GmbH a German Corporation	)	
	)	CIVIL ACTION NO.
Plaintiff,	)	SA-04-CA-807
	)	
v.	)	
	)	
HEMOCUE AB, a Swedish Corporation, and HEMOCUE, INC., a California Corporation,	)	
	)	
	)	
Defendants.	)	

ORDER OF CONSOLIDATION

Having reviewed the complaint in this action, as well as the pleadings in cause number SA-03-CA-1080-OG, it is apparent that both lawsuits have been filed against the same Defendants and both lawsuits seek declaratory relief pertaining to U.S. Patent Number 5,674,457, its validity or invalidity and infringement or non-infringement thereof.

It is therefore ORDERED that Cause No. SA-04-CA-807-OG is hereby CONSOLIDATED with Cause No. SA-03-CA-1080-OG, and all further proceedings shall be conducted under Cause No. SA-03-CA-1080-OG.

SIGNED and ENTERED this 6 day of October, 2004.

ORLANDO L. GARCIA
UNITED STATES DISTRICT JUDGE

United States Patent [19]

Lilja et al.

[11] **4,088,448**

[45] **May 9, 1978**

[54] **APPARATUS FOR SAMPLING, MIXING THE SAMPLE WITH A REAGENT AND MAKING PARTICULARLY OPTICAL ANALYSES**

[76] Inventors: **Jan Evert Lilja**, Frodes vag 17, 291 65 Kristianstad; **Sven Erik Lennart Nilsson**, Hasselvagen 17, Kristianstad, both of Sweden

[21] Appl. No.: **724,054**

[22] Filed: **Sep. 16, 1976**

[30] **Foreign Application Priority Data**

Sep. 29, 1975 Sweden 7510863

[51] **Int. Cl.**[2] **G01N 33/16; G01N 21/24**

[52] **U.S. Cl.** ..... **23/259**; 23/230 B; 23/253 TP; 23/253 R; 128/2 F; 356/246

[58] **Field of Search** ..... 23/253 R, 259, 253 TP, 23/230 B; 356/246; 128/2 F

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,464,800	9/1969	Gerade	23/259
3,497,320	2/1970	Blackburn et al.	23/253 R
3,582,285	6/1971	Hamilton	356/246 X
3,620,676	11/1971	Davis	23/253 TP
3,640,267	2/1972	Hurtig et al.	128/2 F
3,690,836	9/1972	Buissiere et al.	23/253 TP
3,701,633	10/1972	Davis	23/253 TP
3,732,079	5/1973	Davis	23/253 TP
3,768,978	10/1973	Grubb et al.	123/2 F
3,770,382	11/1973	Carter et al.	356/246 X
3,799,742	3/1974	Coleman	356/246 X
3,811,840	5/1974	Bauer et al.	23/253 TP
3,838,013	9/1974	Bergeron	195/139
3,859,050	1/1975	Hern et al.	23/253 R
3,860,347	1/1975	Jones	356/246
3,951,606	4/1976	Moyer et al.	23/259 X
3,994,594	11/1976	Sandrock et al.	356/246

FOREIGN PATENT DOCUMENTS

2,103,841	10/1972	Germany.
2,422,260	11/1975	Germany.
1,307,704	2/1973	United Kingdom.
1,353,632	5/1974	United Kingdom.

OTHER PUBLICATIONS

Bowman et al., "Capillary-Tube Scanner for Mechanized Microbiology", Science, vol. 158, 1967, pp. 78–83.

Primary Examiner—R.E. Serwin
Attorney, Agent, or Firm—Beveridge, DeGrandi, Kline & Lunsford

[57] **ABSTRACT**

A cuvette for sampling with a cavity which is defined by two planar surfaces which are placed or can be placed at a predetermined distance from one another. A reagent is contained in the cavity in an amount which is exactly determined in relation to the volume of the cavity. The sample is drawn into the cavity preferably by capillary force and mixed with the reagent therein spontaneously or by vibration, whereupon optical analysis takes place directly through the two planar surfaces.

16 Claims, 15 Drawing Figures

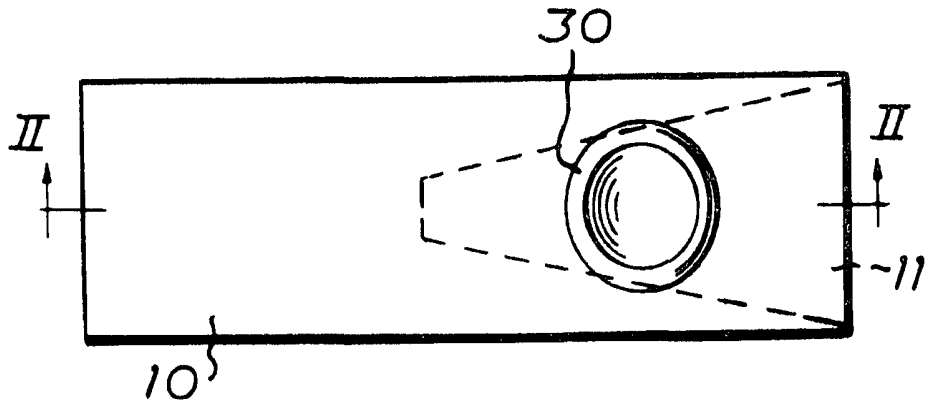


FIG.1

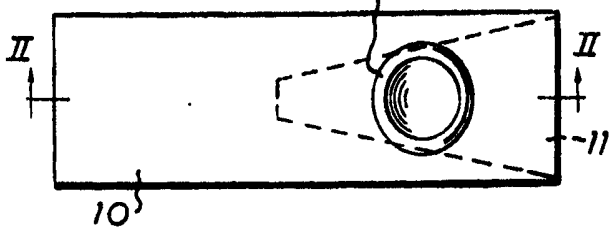


FIG.2

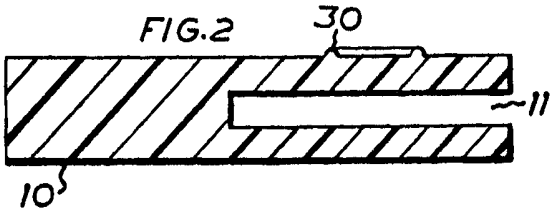


FIG.3

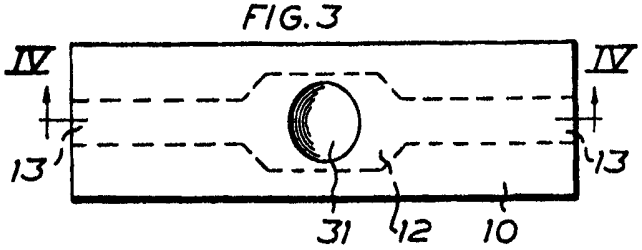


FIG.4

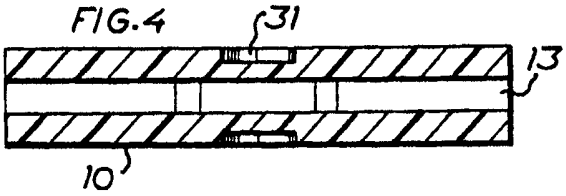


FIG.5

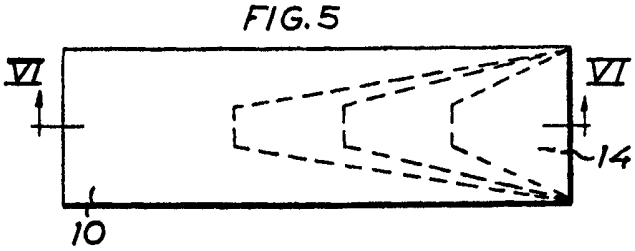


FIG.6

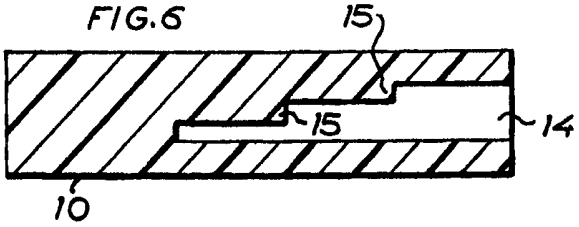


FIG.7

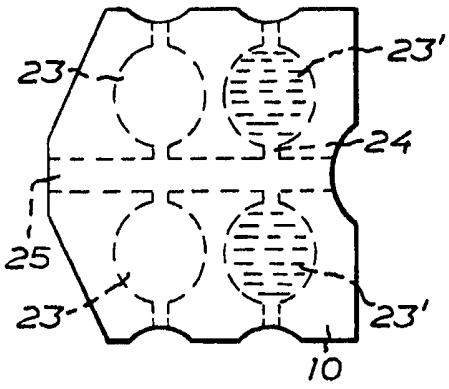


FIG.8

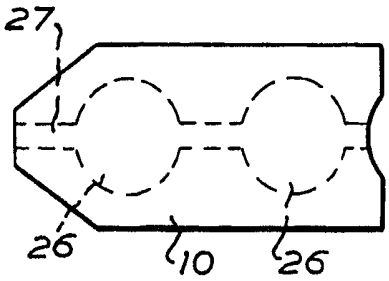


FIG.9

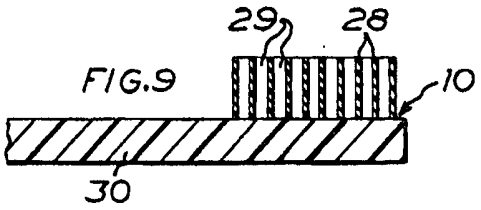


FIG.11

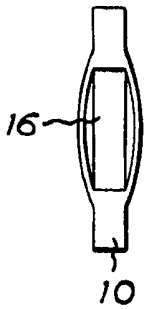


FIG.10

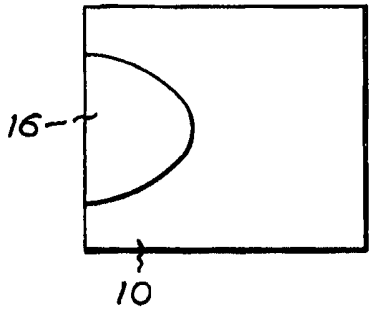


FIG.12

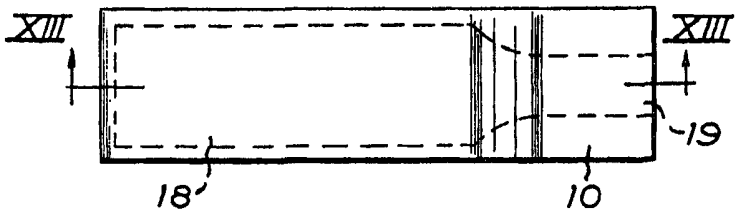


FIG.13

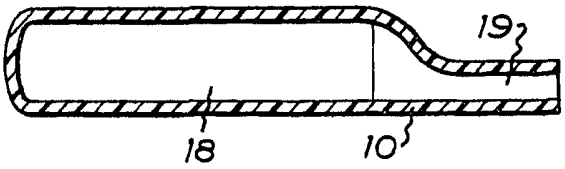


FIG.14

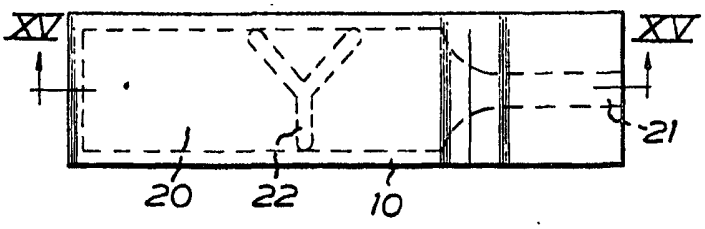
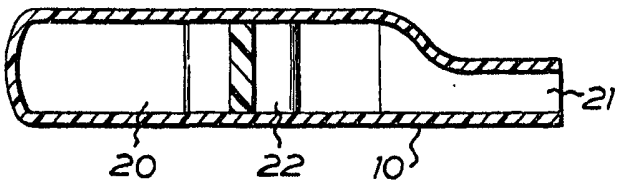


FIG.15



APPARATUS FOR SAMPLING, MIXING THE SAMPLE WITH A REAGENT AND MAKING PARTICULARLY OPTICAL ANALYSES

This invention relates to an apparatus for sampling, mixing the sample with a reagent and directly making particularly optical analyses of the sample mixed with the reagent.

In making wet-chemical analyses a sample is normally measured with a single-use pipette while the reagent is measured in a vessel with a pipette or dispensor. The reagent mostly has a relatively large volume, which results in a relatively small error on measuring the volume, whereas the volume of the sample normally is small, which implies a high dilution after mixing of reagent and sample. To attain a high precision the sample volume must therefore be measured with great exactitude. The sample and reagent mixture is transferred after the reaction time, if any, to an optical measuring cuvette for measurement.

The object of the present invention is to overcome the disadvantages prevailing in analyses of this kind.

To this end there is provided a measuring cuvette which is characterized in that it comprises a body having at least one cavity into which the sample can be drawn, in that the reagent is accommodated in the cavity in an amount predetermined in relation to the volume of said cavity, and in that two opposed planar surfaces defining the cavity are placed or adapted to be placed at a predetermined distance from each other.

The invention permits sampling of a liquid, mixing and chemically reacting it with a suitable reagent, for instance for colour development, in the same vessel as the one used for the subsequent measurement. The invention thus simplifies the sampling procedure, reduces the number of utensils and in most cases, depending upon the type of analysis, considerably improves the exactitude of the analysis by making the analysing procedure independent of the operating technique of the operator making the analysis. Compared with the conventional manual procedures, the gain of time is also considerable.

According to the invention the cuvette may be in the form of an optical measuring cuvette of short light path, which is primarily intended for directly sucking up a sample by the capillary force arising between the walls defining the cavity of the cuvette, by vacuum or by gravity. The cuvette cavity contains an exactly determined amount of reagent, preferably in solid form. The remaining volume of the cuvette cavity is filled with sample liquid. This gives a definite volume relation of the sample liquid to the reagent. The sample liquid is meant to dissolve the reagent, which implies that two earlier manual measuring procedures are replaced by one in which the manufacturing exactitude will be determinative of the measuring exactitude.

The reagent recipe is so formulated that the dissolution of the reagent does not take place too rapidly. An increased dissolution rate is obtained by means of vibratory mixing. The dissolution rate can also be influenced if the reagent is coated with a suitable difficulty soluble substance, which in turn entails the possibility of carrying out chemical reactions in several steps and of separating sensitive reagents.

Mixing suitably takes place by vibration since the liquid layer is so thin that the conventional mixing procedures are ineffective. Mixing is most simply carried out in a photometer but can also be carried out in a separate vibrator. The optimum vibration frequency and amplitude to a certain extent are dependent upon the physical configuration and location of the measuring cuvette. If use is made of a separate vibrator a standard photometer can be utilized on making optical analyses.

The invention shall be described more in detail in the following with reference to the accompanying drawings which schematically and on a large scale illustrate several embodiments. In the drawings:

FIG. 1 shows a measuring cuvette according to the invention;

FIG. 2 shows a section on the line II—II in FIG. 1;

FIG. 3 shows a modified embodiment of the cuvette;

FIG. 4 shows a section on the line IV—IV in FIG. 3;

FIG. 5 shows another embodiment of the measuring cuvette according to the invention;

FIG. 6 shows a section on the line VI—VI in FIG. 5;

FIG. 7 shows an embodiment in which the cuvette has parallel-connected cavities;

FIG. 8 shows an embodiment in which the cuvette has series-connected cavities;

FIG. 9 shows a further embodiment in which the cuvette has a plurality of cavities in the form of channels;

FIG. 10 shows a cuvette with somewhat outwardly bulging walls;

FIG. 11 shows the cuvette according to FIG. 10 from one end;

FIG. 12 shows a cuvette having an inlet channel;

FIG. 13 shows this cuvette in section on the line XIII—XIII;

FIG. 14 shows a cuvette having a spacing element; and

FIG. 15 shows a section on the line XV—XV in FIG. 14.

The cuvette illustrated in the drawings comprises a body 10 of glass or plastics which is preferably transparent to permit making optical analyses. According to FIGS. 1 and 2 the body 10 has a cavity 11 which is intended to accommodate a liquid sample and the dimension of which is such that it can be filled by capillary force or, in some cases, by gravity. The dimensions of the cavity are exactly determined, in particular the distance between the walls defining the cavity.

The cavity 11 of the cuvette is supplied with a reagent (that is, an agent to react with the sample drawn into said cavity) by evaporation, freeze-drying, spraying, screen printing or in another suitable manner according to the manner in which the cuvette is manufactured. The amount of reagent is thoroughly regulated in dependence on the size of the cavity. By coating the reagent the dissolution rate thereof in the sample can be controlled, for instance for the dissolution of reagents in a definite sequence, which is suitable in making analyses in several reaction steps, or for the isolation of sensitive reagents. After a reagent has been deposited in the cavity the cuvette is ready for use. When the cuvette illustrated in FIG. 1 is to be used the outwardly open side of the cavity is brought in contact with the liquid sample to be examined, whereby said sample penetrates into the cavity 10 and there mixes with the reagent either spontaneously or with the aid of, for instance, a vibrator which may be a separate unit or part of the analysing apparatus, for instance a photometer, in which the sample is to be analysed. The sample is then placed in the analysing apparatus, and the analysis is carried out.

As will appear from FIGS. 1 and 2 an annular bead 30 is disposed on the outer side of the upper wall defining the cavity (see FIG. 2). This bead 30 has for its object to protect the enclosed portion of the wall against being scratched or otherwise damaged when the cuvette is handled before making the analysis.

FIGS. 3 and 4 show a modified embodiment of the cuvette 10 which has a cavity 12 and two channels 13 which extend from opposite sides of the cuvette and open into the cavity 12. Thus a sample can here be drawn straight through the cuvette, which may be advantageous in certain cases. A recess 31 having a planar bottom is arranged in each of the opposite walls of the cuvette above the cavity 12. Said recesses are situated opposite one another and serve the same purpose as the annular bead 30 in the foregoing embodiment.

The cuvette according to FIGS. 5 and 6 has a cavity 14 of varying depth, which has been realized by stepping one of the surfaces of the cavity to form levels 15 spaced different distances from the opposite surface. The number of such levels can be varied and the height difference between the levels will be determinative for the measuring exactitude. The outermost cavity can serve as a receiving cavity which is devoid of reagent and from which the sample can be drawn at a suitable rate into the other cavities. Of course, a bead 30 or recess 31 according to the two preceding embodiments may be disposed above one or more levels.

The cuvette as shown in FIG. 7 has four parallel-connected cavities 23, 23', which are linked to a common channel 25 by branch channels 24 which continue on the opposite side of the cavities and open into the atmosphere to prevent air inclusions in the cavities when samples are drawn thereinto. The cavities 23, 23' can contain reagents of the same or different kinds for making control analyses and various kinds of analyses, respectively. In a preferred embodiment the cavities 23' instead of containing a reagent deposited on the walls thereof, may contain a gel, for instance, one gel of a given type in the upper cavity of the figure and another gel in the lower cavity thereof. This will provide the advantage that a sample can be caused to react with two different reagents in the cavities 23 while particles of a size determined by the choice of gel diffuses into the gel in the respective cavity 23' to react with a specific reagent contained in the gel.

FIG. 8 shows two series-connected cuvette cavities 26 into which a sample is drawn through a channel 27. Said cavities may contain reagents of the same kind for making control analyses or of different kinds for making different analyses. As in the previous embodiment one cavity may contain a reagent and the other cavity — the inner one — a gel.

The embodiment according to FIG. 9 differs from those already described in that the cuvette is formed by a supporting plate 30 on which is fixed, for instance by gluing, a rigid porous material 28 with channels or cavities 29 extending at right angles to the plane of the supporting plate. Same as in the preceding embodiments, a reagent cr reagents are deposited in said channels or cavities 29. In this case the distance between the end surfaces of the channels or cavities is of importance as the analysis here takes place in the longitudinal direction of the channels or cavities. Of course, like in the preceding examples, the channel volume is of importance.

The cuvette shown in FIGS. 10 and 11 is made of a more elastic plastic material than the preceding cuvettes and has a cavity 16 which — as will appear from the end view in FIG. 11 — is defined by outwardly bulging walls which by a mechanical device are compressed to take a predetermined distance from each other at the subsequent analysis. The cuvette according to FIGS. 12 and 13 likewise consists of elastic plastic material and has a cavity 18 with an inlet channel 19. In this case also the walls defining the cavity are compressed at the subsequent analysis. The thickness tolerance of the cuvette material will be decisive of the measuring exactitude both in the former and the latter apparatus, as will the stability of the device by means of which the walls are compressed.

The cuvette shown in FIGS. 14 and 15 is also made of elastic material and formed with a cavity 20 and inlet channel 21 in the same way as the cuvette shown in FIG. 12. But the cuvette in FIGS. 14 and 15 has an internal spacing element 22 which can serve to determine the interstice to which the surfaces defining the cavity 20 can be compressed, and to support the reagent. Besides, the spacing element 22 may have a mixing function if it is made of ferromagnetic material and mixing is realized by placing the cuvette in a variable magnetic field.

The cuvettes illustrated in FIGS. 10–15 can be filled by capillary force, gravity or vacuum.

In case the sample drawn into the cuvette shall not be immediately analysed or in case the analysis takes a long time, means may be provided to close the outwardly facing open end of the cavity and inlet channel, respectively. Such means may be a plastic hood which is pulled over the opening, or a material of suitable consistency into which the cuvette is dipped and which immediately seals the opening.

If desired, the cuvette can readily be provided with means such as projections and recesses which operate measuring equipment at the subsequent analysis.

The measuring cuvette described is useful for making analyses of the most varying kinds. It has, however, been found to be of special advantage in the determination of hemoglobin, where it has proved possible to reduce the error margin to an absolute minimum. If the cuvette has a receiving cavity into which the sample is drawn by vacuum, gravity or capillary force and from which the sample is supplied, by capillary force, to a plurality of cavities containing different reagents and/or gels a large number of analyses can be rapidly made particularly if the cuvette is fed into an automatic measuring apparatus which is controlled by specially designed cuvette parts.

As will appear from the foregoing, the cuvette according to the invention in an extremely simple way permits making analyses which without exception have hitherto been difficult and time-consuming and necessitated great skill of the operator to avoid errors.

What we claim and desire to secure by Letters Patent is:

1. A cuvette for sampling a fluid, mixing the sample with a reagent, and directly making optical analyses of the sample mixed with the reagent, comprising a body member including two planar surfaces defining an optical path and placed at a predetermined distance from one another to determine the optical path length and to define at least one cavity having an inlet communicating the at least one cavity with the exterior of the body member, the at least one cavity having a predetermined fixed volume, the predetermined distance being effec-

tive to permit the sample to enter the cavity by capillary force; and a reagent coated on the cavity surface.

2. A cuvette as claimed in claim 1, wherein the reagent is in the form of a solid material deposited on the walls of the cavity by evaporation, freeze-drying, spraying or screen printing.

3. A cuvette as claimed in claim 1, wherein the reagent is in the form of a semi-solid material, particularly a gel.

4. A cuvette as claimed in claim 1, wherein the cuvette has a receiving space communicating with the at least one cavity and with the inlet and from which the at least one cavity withdraws the sample by capillary force.

5. A cuvette as claimed in claim 1, wherein said body member has a plurality of cavities arranged in parallel or in series, said cavities containing the same or different reagents.

6. A cuvette as claimed in claim 1, wherein said body member is adapted for mixing of the sample with the reagent by actuation from the outer side of the body member.

7. A cuvette as claimed in claim 6, wherein said body member is adapted for mixing actuation by vibration.

8. A cuvette as claimed in claim 1, wherein at least one of the walls defining the cavity of the body member is stepped with exactly determined height differences between the different steps.

9. A cuvette as claimed in claim 1, wherein the reagent or reagents are coated for regulation of the dissolution rate.

10. A cuvette as claimed in claim 1, wherein the at least one cavity is oriented with its longitudinal direction at right angles with the body member planar surfaces.

11. A cuvette as claimed in claim 1, made from transparent plastic material or glass.

12. A cuvette for sampling a fluid, mixing the sample with a reagent, and directly making optical analyses of the sample mixed with the reagent, comprising a body member including two flexible surfaces and means joining the two surfaces to define at least one cavity having an inlet communicating the at least one cavity with the exterior of the body member, the joining means separating the flexible surfaces by a dimension effective to permit the sample to enter the at least one cavity by capillary force, the two flexible surfaces being adapted to be placed at a predetermined distance from one another by actuation from the outer side of the body member to define an optical path having a predetermined optical path length and to provide a predetermined volume in the cavity; and a reagent coated on the cavity surface.

13. A cuvette as claimed in claim 12, further comprising a spacing element disposed in the cavity to determine the distance between the walls of the cavity upon actuation of said walls from outside.

14. A cuvette as claimed in claim 13, wherein the reagent is carried by the spacing element.

15. A cuvette as claimed in claim 13, wherein the spacing element is of ferromagnetic material to provide mixing of the sample and the reagent when the cuvette is placed in a variable magnetic field.

16. A cuvette as claimed in claim 12 further comprising a reagent in the cavity in the predetermined amount.

* * * * *

United States Patent [19]

Lilja et al.

[11] **Patent Number: 4,654,197**

[45] **Date of Patent: Mar. 31, 1987**

[54] **CUVETTE FOR SAMPLING AND ANALYSIS**

[75] Inventors: **Jan E. Lilja**, Kristianstad; **Sven E. L. Nilsson**, Helsingborg, both of Sweden

[73] Assignee: **Aktiebolaget Leo**, Helsingborg, Sweden

[21] Appl. No.: **660,466**

[22] Filed: **Oct. 12, 1984**

[30] **Foreign Application Priority Data**

Oct. 18, 1983 [SE] Sweden 8305704

[51] Int. Cl.[4] **G01N 21/78**; G01N 27/28; G01N 33/52

[52] U.S. Cl. **422/56**; 204/403; 422/58; 422/61; 435/810

[58] Field of Search 422/58, 100, 102, 61, 422/56, 57; 436/165, 172, 166, 178; 356/246; 204/403; 435/810

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,420,205	1/1969	Morison	422/56 X
3,787,291	1/1974	Deuringer et al.	204/403 X
3,865,548	2/1975	Padawer	422/61 X
3,904,373	9/1975	Harper	436/166 X
4,003,707	1/1977	Lubbers et al.	436/172
4,008,448	5/1978	Lilja et al.	356/246 X
4,042,335	8/1977	Clement	422/56
4,200,110	4/1980	Peterson et al.	436/163 X
4,233,029	11/1980	Columbus	422/55 X
4,234,316	11/1980	Hevey	436/166
4,269,804	5/1981	Kring	422/61 X
4,323,536	4/1982	Columbus	422/58 X
4,387,164	6/1983	Hevey et al.	436/166 X
4,418,148	11/1983	Oberhardt	204/403 X

FOREIGN PATENT DOCUMENTS

0078225	5/1983	European Pat. Off.	436/166
2039039	7/1980	United Kingdom	436/166

OTHER PUBLICATIONS

Romette et al., Clinica Chimica Acta, 95 (1979), 249–253.

Mascini et al., Analytical Chemistry, vol. 49, No. 6, May 1977, pp. 795–798.

Vadgama, Journal of Medical Engineering & Technology, vol. 5, No. 6, Nov. 1981, pp. 293–298.

Primary Examiner—Arnold Turk

Attorney, Agent, or Firm—Burgess, Ryan & Wayne

[57] **ABSTRACT**

Disposable cuvette for essentially simultaneous sampling of a fluid and analyzing the sample. The new cuvette comprises a body member having at least one cavity defined by surrounding walls, into which cavity the sample is permitted to enter by capillary force through an inlet communicating said cavity with the exterior of the body member. According to the invention, the cuvette is characterized in that at least a portion of the walls facing the cavity consists of a semipermeable membrane, optionally with an integrated electrode and/or sensor system, and that at least one reagent or reagent system is incorporated in the cuvette.

12 Claims, 10 Drawing Figures

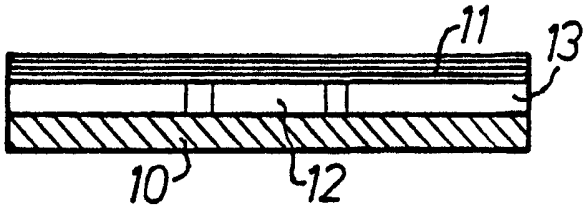


FIG.1

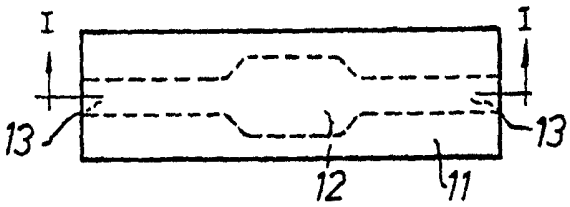


FIG.2

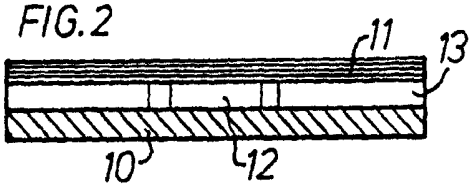


FIG.2a

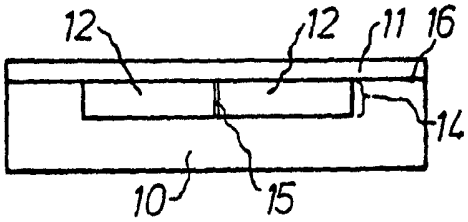


FIG.2b

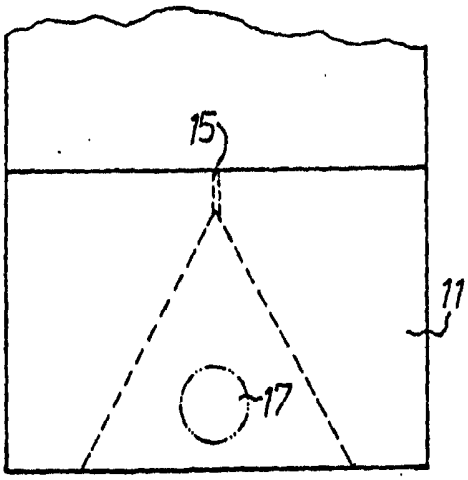


FIG. 3

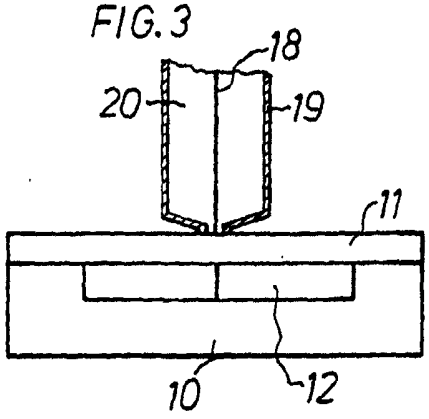


FIG. 4

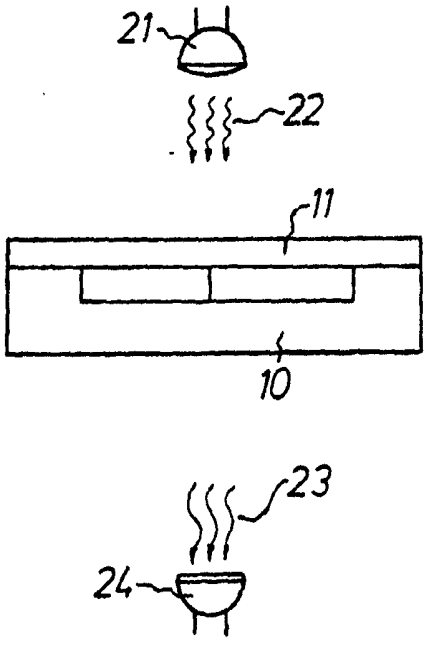


FIG.5

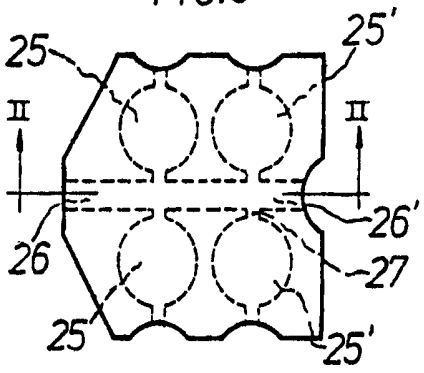


FIG.5a

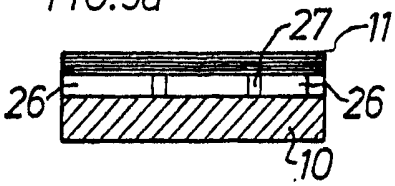


FIG.6

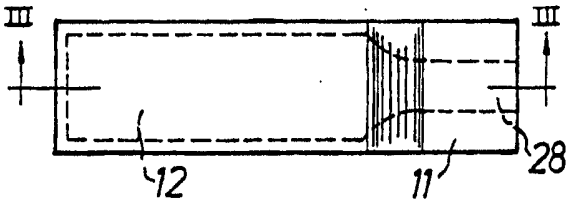
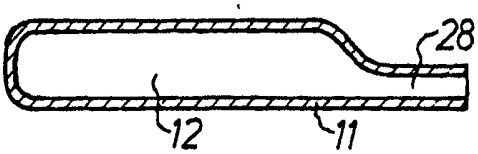


FIG.6a



CUVETTE FOR SAMPLING AND ANALYSIS

The present invention concerns a disposable cuvette for essentially simultaneous sampling of a fluid and analyzing the sample.

A cuvette for sampling a fluid, mixing the sample with a reagent and directly making optical analyses of the sample mixed with the reagent is previously known from U.S. Pat. No. 4,088,448. This cuvette comprises a body member including two planar surfaces defining an optical path and placed at a predetermined distance from one another to determine the optical path length and to define a cavity having an inlet communicating said cavity with the exterior of the body member. The cavity has a predetermined fixed volume, and the predetermined distance permits the sample to enter the cavity by capillary force. Furthermore, a reagent is coated on the cavity surface.

This known cuvette has serveral advantages when compared with conventionally used devices. It permits sampling of a liquid, mixing and chemically reacting it with a suitable reagent e.g. colour development in the same vessel as the one used for the subsequent measurement. The cuvette disclosed in U.S. Pat. No. 4,088,448 thus simplifies the sampling procedure, reduces the number of utensils and—in most cases, depending on the type of analysis—considerably improves the accuracy of analysis by making the analyzing procedure independent of the operating technique of the operator making the analysis.

The present invention concerns an improvement of this known cuvette.

To this end, there has been developed a disposable cuvette for essentially simultaneous sampling of a fluid and analyzing the sample, comprising a body member having at least one cavity defined by surrounding walls, into which cavity a sample is permitted to enter by capillary force through an inlet communicating said cavity with the exterior of said body member, the cuvette being characterized in that at least a portion of the walls facing the cavity consists of a semipermeable membrane, optionally with an integrated electrode and-/or sensor system, and that at least one reagent or reagent system is incorporated in the cuvette.

One advantage of the improved cuvette is that it can be used for other types of measurements than optical analyses, which makes it applicable to analyses within a much broader range than the cuvette according to U.S. Pat. No. 4,088,448. Thus, according to the present invention, the measurement can be carried out by using different electrodes, the surfaces of which are pressed against the exterior surface of the semipermeable membrane. Furthermore, optical instruments may be used. Within the scope of the present invention are also electrode or sensor systems integrated with, i.e. applied on or incorporated in, the semipermeable membrane material.

Another very important advantage as compared with the previously known cuvette is that the use of membranes makes it possible to separate sample media from reagent media, and interferences originating from substances, unsuitable pH; unsuitable redox environment etc. can be avoided. Thus, two or more reaction systems, which are incompatible, may be included in the new cuvette, as the semipermeable membrane acts as a barrier which prevents a component, e.g. a reagent contained in the cavity from entering and disturbing the

reaction(s) in the membrane(s), and vice versa. This second advantage makes the field of application for the present cuvette even broader and useful for a wide variety of different analyses.

Thus, the additional advantages according to the present invention emanate from the use of the semipermeable membrane and the possibility of combining this membrane with external or internal electrodes.

Analyses based on the use of semipermeable membranes and electrodes are known in the art. However, using known techniques, difficulties are encountered in the handling of the sample, electrodes which often are sensitive to contamination, may be contaminated, evaporation of the sample may occur, and the sample may be subjected to the influence of different types of gases, such as the oxygen of the air. All these problems can be avoided by using the cuvette according to the present invention.

According to the present invention, the body member may consist of glass or polymeric material. It is also quite possible to make the whole body member or one wall thereof of the semipermeable material which in this case preferably should be self-supporting. If not essentially self-supporting, the membrane could be used as a coating on the surface of the body member facing the cavity. The reagent, if any, coated on at least a portion of the body (body member) surface facing the cavity may be deposited by evaporation, freeze-drying, spraying or screen-printing, as known in the art.

The semipermeable membrane may be in the form of one separate membrane layer or two or more separate layers joined to each other to form a composite membrane. The various reagents may be coated on the membrane surface facing the cavity and applied thereto by evaporation, freeze-drying, spraying or screenprinting, etc. It is also possible to have the reagents deposited as a layer on separate surfaces of the membrane in such a way that this layer becomes an intermediate layer in the finished composite membrane. One or more such layers may be present. The semipermeable membrane may also be prepared in such a manner that the reagent or reagents are dispersed or dissolved throughout the whole membrane or one or more layers thereof. Another possibility is to prepare the membrane material in such a manner that the reagent molecules are covalently bound to the polymer molecules of the semipermeable membrane.

The semipermeable membrane material is chosen in dependence on the kind of analysis to be performed and may be determined by a person skilled in the art. The membrane material might be hydrophilic or hydrophobic. Examples of different material which can be used according to the present invention are Teflon ®, silicon rubber, polyacrylates, polyvinyls, collagen and even crosslinked enzymes, etc.. Various substances could be incorporated in the membrane to give special selective properties, to perform a chemical reaction, etc. Including specific crown ethers in a polyvinyl membrane gives a membrane with selective properties for alkaline ions. Including glucose oxidase in a membrane makes it possible to measure glucose by the production of hydrogen peroxide or the decrease in oxygen concentration.

The membrane may selectively permit penetration of only or essentially the substance/ion, which is relevant-/interesting, and which can be detected by, for example, an electrode on the external surface of the membrane. Furthermore, the membrane may function as a

discriminator in which only molecules/ions below a certain size can move freely.

To perform measurements with electrodes on or in the improved cuvette, the membrane acts as a semipermeable barrier (with or without selective properties) which prevents the electrodes from being contaminated by the sample medium and/or the reagent. The membrane could participate in a chemical reaction through incorporated reagents and/or selectively permit free passage for the substances to be determined at the electrode.

The electrode to be used according to the present invention may be a conventional potentiometric, i.e. ion-selective or amperometric electrode which, together with the semipermeable membrane of the cuvette, functions as an enzyme electrode or biosensor of the type described in e.g. P. Vadgama, Journal of Medical Engineering & Technology, Vol. 5, No. 6, 1981, 293–298.

Examples of electrodes to be used with the membrane cuvette of the present invention are conventional electrodes, such as a glass electrode (pH), a platinum, gold, or carbon electrode, and other more exclusive electrodes, such as solid state devices of the type CHEMFET or ISFET with their associated electronic parts.

An example of a platinum/silver-silver chloride electrode system together with a composite membrane for determining glucose by amperometric measurement of consumed oxygen is given in a paper by Jean-Louis Romtte, B. Fromment & D. Thomas (Clin. Chim. Acta, 95 (1979) 249–253).

An example of glass electrode application together with a composite membrane for determining urea by pH-measurement of produced ammonia is disclosed in a paper by M. Mascini and G.G. Guilbault (Anal. Chem., Vol. 49, No. 6, May 1977, 795–798).

As regards optical analyses to be performed with the present cuvette, there are two main possibilities:

(A) the colour develops in the cavity;

(B) the colour develops within the membrane.

In (A), the two main surfaces of the cavity must have a predetermined or a determinable distance between one another to make it possible to determine the optical path length. The determinable distance may be obtained by applying an external force to the surface of an essentailly elastic membrane until the movement of the membrane is stopped against a spacer of predetermined thickness inserted in the cavity.

In (B), the colour developing part (layer) of the membrane or the entire membrane must be of a predetermined thickness to accomplish a determined optical path length.

A practical example of (B) is a cuvette designed to perform an analysis of urea in serum or urine. The cavity contains urease and an alkaline buffer system in dry form which, when dissolved in the sample medium, give free ammonia from urea, and the membrane incorporates an indicator (=a reagent) for ammonia. The membrane is manufactured from a polymer, the hydrophobicity of which is sufficienty high to prevent the alkaline buffer from interfering with the indicator, but is permeable to ammonia. The indicator is a solvent soluble pH-indicator with an indicator interval within the acid range.

Different approaches to the analyses may be made by using different types of electrodes, different types of membranes and different reaction routes, as recognized by a person skilled in the art.

The invention will be described in more detail below, reference being had to the accompanying drawings which illustrate schematically and on a large scale a number of embodiments. In the drawings:

FIG. 1 shows an elevational view of a measuring cuvette according to the invention.

FIG. 2 shows a section on line I—I in FIG. 1;

FIG. 2*a* shows an elevational view of a modified embodiment of the cuvette;

FIG. 2*b* shows a view of top plan view of the cuvette FIG. 2*a*;

FIG. 3 shows an elevational view of the cuvette according to FIGS. 2 and 2*a* in contact with a measuring electrode;

FIG. 4 shows an elevational view of the cuvette according to FIGS. 2 and 2*a* adapted for optical measurement;

FIG. 5 shows a plan view of an embodiment in which the cuvette has parallel-connected cavities;

FIG. 5*a* shows a section on line II—II of FIG. 5;

FIG. 6 shows a plan view of a further modified embodiment of the cuvette;

FIG. 6*a* shows a section on line III—III of FIG. 6.

The cuvette illustrated in FIGS. 1 and 1*a* comprises a body wall 10 of glass or polymeric material, and a body wall 11 of semipermeable membrane material. The walls define a cavity 12 which is intended to accommodate a liquid sample and the dimension of which is such that it can be filled by capillary force. Two channels 13 extend from opposite sides of the cuvette and open into the cavity 12. Thus, a sample can here be drawn straight through the cuvette, which may be advantageous in certain cases. The cavity 12 might be supplied with a reagent (that is an agent to react with the sample drawn into said cavity) by evaporation, freeze-drying, spraying, screen-printing or in another suitable manner according to the manner in which the cuvette is manufactured.

The wall 11 of semipermeable membrane material may be manufactured in such a manner that a reagent system is incorporated in the memebrane, e.g. dispersed or dissolved therein. It is also possible to manufacture the membrane in such a way that the components (molecules) of the reagent system are covalently bound to the polymers constituting the membane material. Another possibility is to build up a semipermeable membrane of two or more layers and apply the reagent systems as intermediate layers between two adjacent membrane layers. One or more such layers and intermediate layers may be present. All types of combinations of incorporation of the reagent system apparent to those skilled in the art fall within the scope of the present invention.

In FIG. 2*a*, 10 is a body wall of polymeric supporting material. 11 is a semipermeable membrane optically composed of several layers. 12 is the cavity accommodating the sample. Elevations 14 determine the optical path length. When the sample is drawn into the cavity 12, air is pressed out through the slit 15. The body wall 10 and the semipermeable membrane 11 are joined together (welded or glued) along the joint 16. The area 17 indicates a suitable measuring zone.

In FIG. 3 a measuring electrode is brought into contact with the semipermeable membrane 11 in the cuvette disclosed in FIGS. 2*a* and 2*b*. In this special embodiment, the electrode consists of a platinum electrode 18 and a reference silver/silver chloride electrode

19. 20 designates the glass body surrounding the platinum electrode 18.

In FIG. 4 cuvette of the FIGS. 2*a* and 2*b* is adapted for optical measuring. Thus, 21 here designates a light source e.g. monochromatic light. 22 indicates the light path towards the cuvette. 23 indicates the light path of unabsorbed light after the cuvette, and 24 is an optical detector.

The cuvette as shown in FIG. 5 has four parallel-connected cavities 25, 25' which are connected to a common channel, 26' by branch channels 27 which continue on the opposite side of the cavities and open into the atmosphere to prevent air inclusions in the cavities when samples are drawn thereinto. Different reactive systems may be included in different cavities and/or the membrane material defining the whole or part of the cavity.

FIG. 5*a* shows a section of the cuvette according to FIG. 5 along line II—II.

The embodiment of the present invention according to FIGS. 6 and 6*a* consists of elastic semipermeable material. The inlet channel 28 communicates the exterior of the cuvette with the cavity 12.

While the invention has been described with reference to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made without departing from the spirit and scope of the invention. Many alternative container designs can be conceived which give the advantageous results herein disclosed.

Further, it is obvious that any analytical procedure can be adapted to the invention herein disclosed. The cuvette is particularly suitable for routine blood chemistry, such as glucose, blood urea nitrogen, albumin, bilirubin, total protein, etc., and numerous other analytical tests.

Accordingly, all substitutions, additions and modifications to which the present invention is readily susceptible, without departing from the spirit and scope of this disclosure, are considered part of the present invention.

What we claim and desire to secure by Letters Patent is:

1. A disposable cuvette for simultaneously sampling a fluid and analyzing the sampled fluid, comprising:

a body member having an exterior and at least one cavity defined by surrounding walls,

an inlet in said walls communicating said cavity with the exterior of the body member and through which said sampled fluid can enter said cavity by capillary forces, at least one of the walls including a semipermeable membrane, said semipermeable membrane having one surface defining a portion of said cavity and an opposite surface forming an external surface of said cuvette, said semipermeable membrane having a predetermined porosity and thereby functioning as a discriminator to permit entry only of molecules/ions up to a certain size, and at least one reagent incorporated in the cuvette in contact with said semipermeable membrane.

2. Cuvette according to claim 1, wherein said semipermeable membrane includes a polymeric material, and molecules of the at least one reagent are covalently bound to the polymers of the semipermeable membrane.

3. Cuvette accoridng to claim 1, wherein said at least one reagent is coated on at least a portion of a surface of said semipermeable membrane facing the cavity.

4. Cuvette according to claim 1, wherein said surrounding walls of said cavity are defined by two spaced planar surfaces, and said two planar surfaces of the cavity define an optical path length.

5. Cuvette according to claim 1, wherein the semipermeable membrane has a predetermined fixed thickness defining an optical path length.

6. Cuvette according to claim 1, further including sensor means applied on the semipermeable membrane for analyzing a sampled fluid.

7. Cuvette according to claim 1, further including sensor means incorporated in the semipermeable membrane for analyzing a sampled fluid.

8. Cuvette according to claim 1, wherein the semipermeable membrane comprises at least one membrane layer.

9. Cuvette according to claim 8, wherein at least one reagent is in contact with at least one membrane layer of the semipermeable membrane.

10. Cuvette according to claim 9, wherein said at least one reagent is dispersed in at least one layer of said semipermeable membrane.

11. Cuvette according to claim 9, wherein said at least one reagent is dissovled in at least one layer of said semipermeable membrane.

12. Cuvette according to claim 9, wherein said at least one reagent is located on said semipermeable membrane.

* * * * *

US00D337388S

United States Patent [19]

Nilsson et al.

[11] Patent Number: Des. 337,388

[45] Date of Patent: ** Jul. 13, 1993

[54] CUVETTE FOR AN OPTICAL ANALYSIS OF LIQUIDS

[75] Inventors: Sven-Erik Nilsson; Jan Lilja, both of Helsingborg, Sweden

[73] Assignee: Hemocue AB, Ängelholm, Sweden

[**] Term: 14 Years

[21] Appl. No.: 669,321

[22] Filed: Mar. 14, 1991

[30] Foreign Application Priority Data

Sep. 14, 1990 [SE] Sweden 901951

[52] U.S. Cl. D24/224; D24/227

[58] Field of Search D24/224; 215/356, 365; 220/500, 23.2, 23.8; 356/246; 435/296; 422/61, 91, 99, 102; 606/160

[56] References Cited

U.S. PATENT DOCUMENTS

D. 263,876	4/1982	Beidler et al.	D24/227 X
D. 278,182	3/1985	Aihara et al.	D24/224
D. 283,728	5/1986	Aihara	D24/224
3,627,432	12/1971	Bergmann	422/102 X
4,119,407	10/1978	Goldstein et al.	422/100 X
4,227,810	10/1980	Sandrock et al.	D24/226 X

Primary Examiner—A. Hugo Word
Assistant Examiner—I. Simmons
Attorney, Agent, or Firm—Kane, Dalsimer, Sullivan, Kurucz, Levy, Eisele & Richard

[57] CLAIM

The ornamental design for a cuvette for an optical analysis of liquids, as shown and described.

DESCRIPTION

FIG. 1 is a side elevational view of a cuvette for an optical analysis of liquids showing our new design;
FIG. 2 is a top plan view thereof;
FIG. 3 is a bottom plan view thereof;
FIG. 4 is an end elevational view thereof;
FIG. 5 is an inverted perspective view thereof; and,
FIG. 6 is a perspective view thereof taken toward the same side as that shown in FIG. 5.

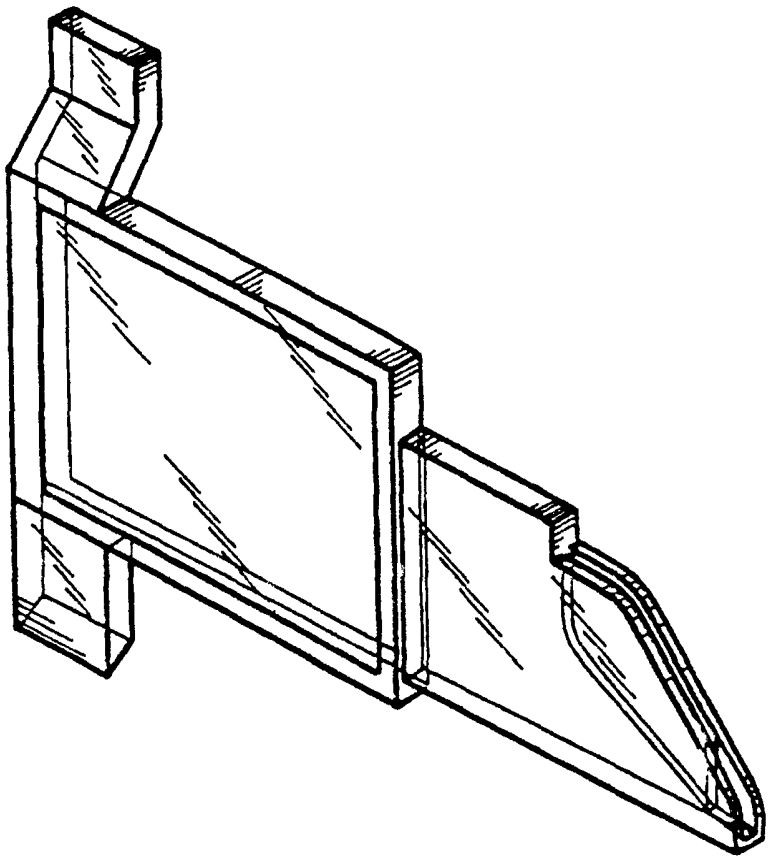


FIG.3

FIG.4

FIG.1

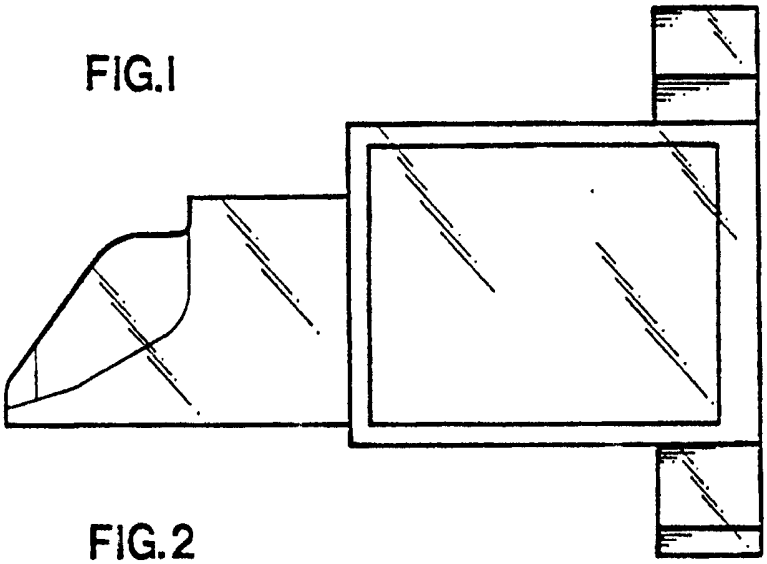


FIG.2

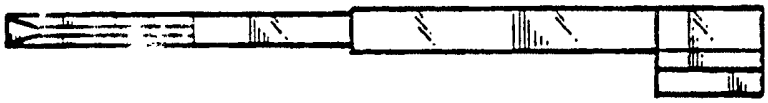


FIG.5

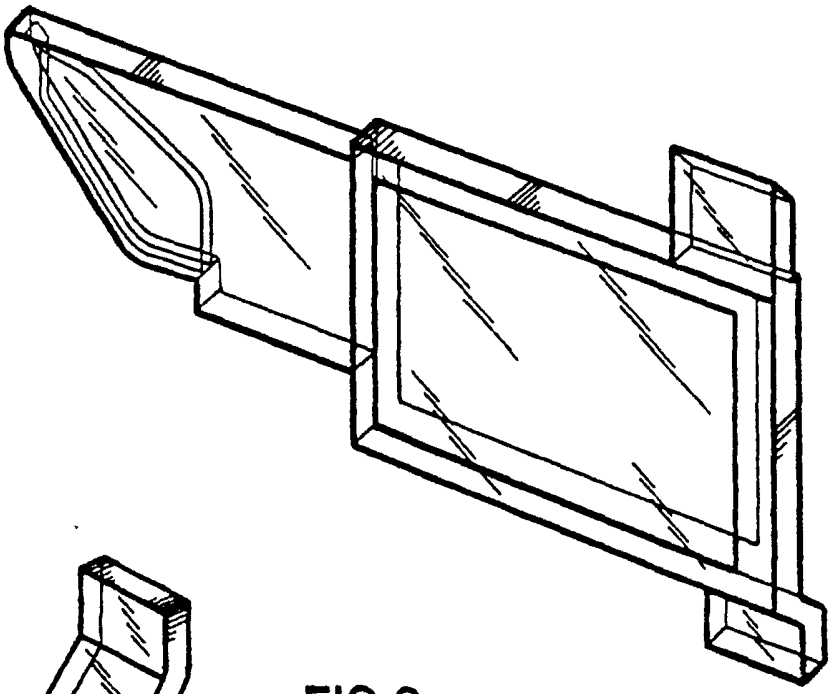
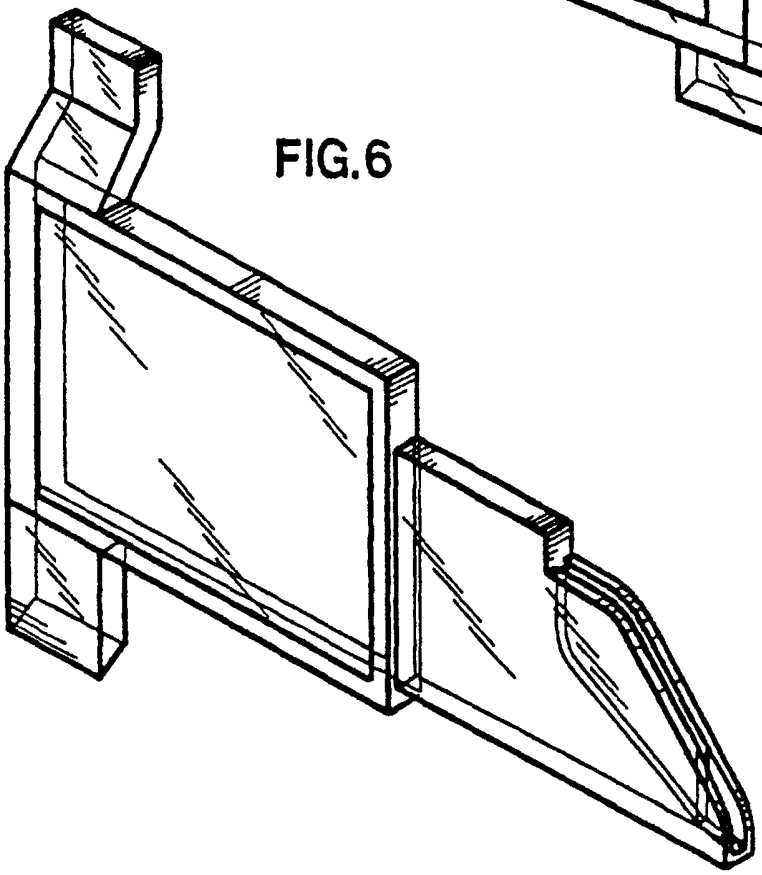


FIG.6



US005286454A

United States Patent [19]

Nilsson et al.

[11] Patent Number: 5,286,454

[45] Date of Patent: Feb. 15, 1994

[54] CUVETTE

[76] Inventors: Sven-Erik Nilsson, Döbeliusvägen 39, S-253 67 Helsingborg; Jan Lilja, Södra Brunnsvägen 63, S-253 68 Helsingborg, both of Sweden

[21] Appl. No.: 768,321

[22] PCT Filed: Apr. 25, 1990

[86] PCT No.: PCT/SE90/00275

§ 371 Date: Oct. 17, 1991

§ 102(e) Date: Oct. 17, 1991

[87] PCT Pub. No.: WO90/13016

PCT Pub. Date: Nov. 1, 1990

[30] Foreign Application Priority Data

Apr. 26, 1989 [SE] Sweden 8911518

[51] Int. Cl.[5] B01L 3/00

[52] U.S. Cl. 422/102; 422/101; 422/72; 422/58; 422/57; 436/177; 436/178

[58] Field of Search 422/102, 72, 57, 58, 422/101; 436/45, 177, 178, 180, 809; 356/246; 494/16-21, 45; 210/206; 435/287, 291, 301, 311

[56] References Cited

U.S. PATENT DOCUMENTS

1,365,183	1/1921	Moffatt	422/102
3,795,451	3/1974	Mailen	422/72
3,799,742	3/1974	Coleman	356/246
3,901,658	8/1975	Burtis et al.	422/72
4,066,512	1/1978	Lai et al.	435/10
4,088,448	5/1978	Lilja	422/57
4,178,345	12/1979	Terk	435/287
4,284,602	8/1981	Kelton et al.	356/246
4,330,206	5/1982	Gausmann et al.	356/246
4,447,546	5/1984	Hirschfeld	422/57
4,462,964	7/1984	Guigan	422/61
4,605,536	8/1986	Kuhnert et al.	422/58
4,654,197	3/1987	Lilja et al.	422/58
4,696,798	9/1987	Timgren	422/102
4,714,590	12/1987	Guigan	422/102
4,756,884	7/1988	Hillman et al.	422/81
4,806,316	2/1989	Johnson et al.	422/58
4,883,763	11/1989	Holen et al.	422/102
4,999,304	3/1991	Robertson	436/177
5,039,617	8/1991	McDonald et al.	422/58

Primary Examiner—Robert J. Warden
Assistant Examiner—Hien Tran
Attorney, Agent, or Firm—Kane, Dalsimer, Sullivan, Kurucz, Levy, Eisele and Richard

[57] ABSTRACT

A cuvette for taking up a fluid and mixing the fluid with a reagent for analyzing the mixture consists of a body (10) of glass or polymeric material having a first cavity (12) in which the fluid can be taken up, preferably by capillary action, through an inlet (13), and at least one further cavity (21) exerting capillary force on fluid which is transported from the first cavity (12) into a reception cavity (17) by subjecting the cuvette to centrifugal force. The further cavity (21) preferably exerts capillary force through a wick (19) which does not extend as far as the bottom of the reception cavity (17), and a capillary channel (20). In those cases where more than one further cavity (21) is provided, each such cavity (21) communicates with a further reception cavity into which the fluid can be transported from the cavity (21) by the exertion of centrifugal force. The cuvette may also have cavities for receiving washing or diluting liquid, which are connected in series or in parallel with the cavity (12).

17 Claims, 2 Drawing Sheets

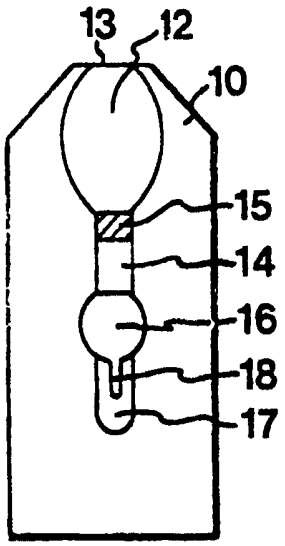


FIG.1

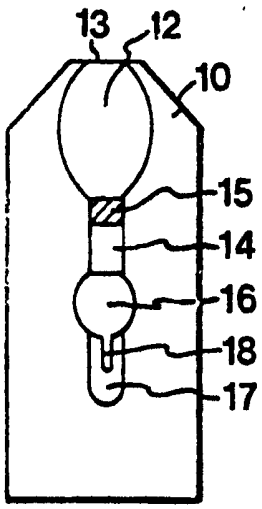


FIG.2

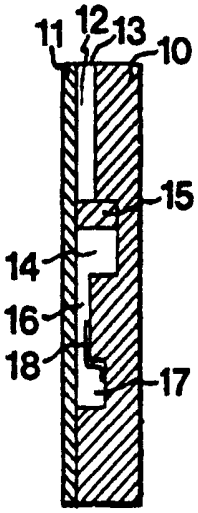


FIG.3

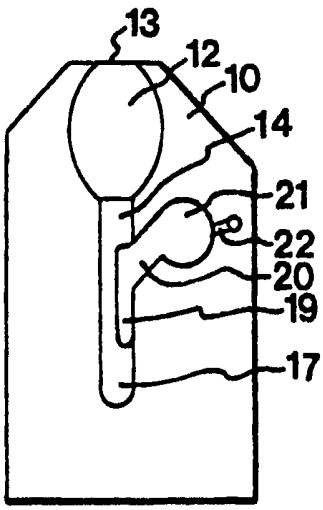


FIG.4

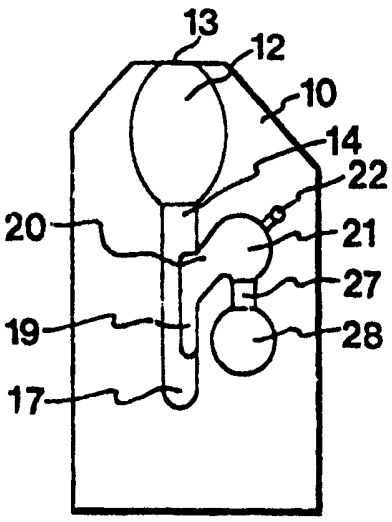


FIG.5

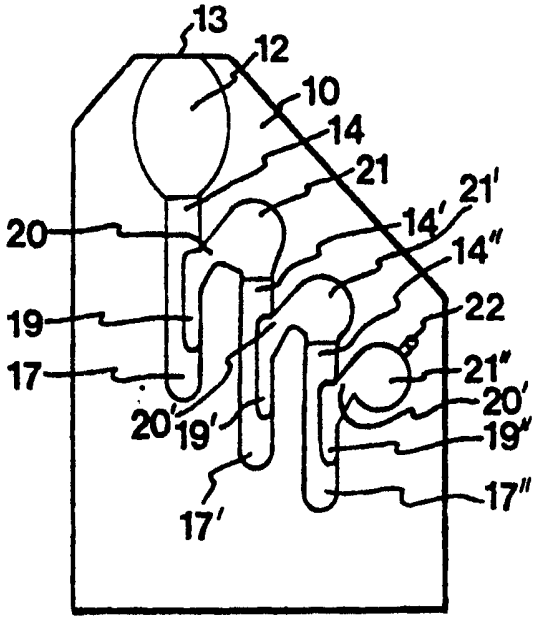


FIG.6

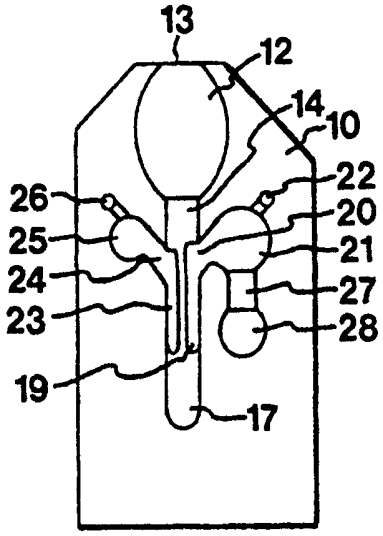


FIG.8

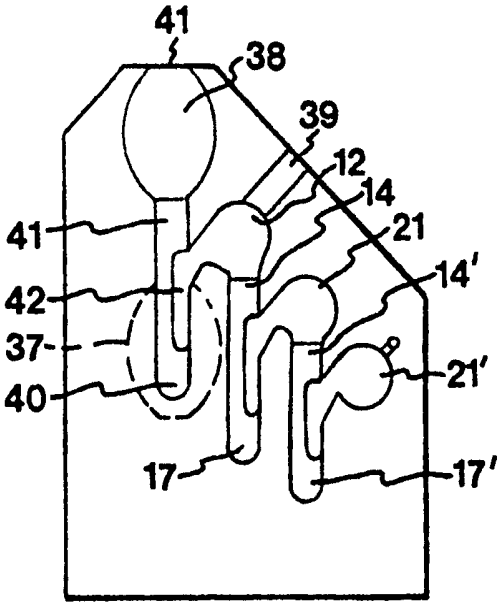
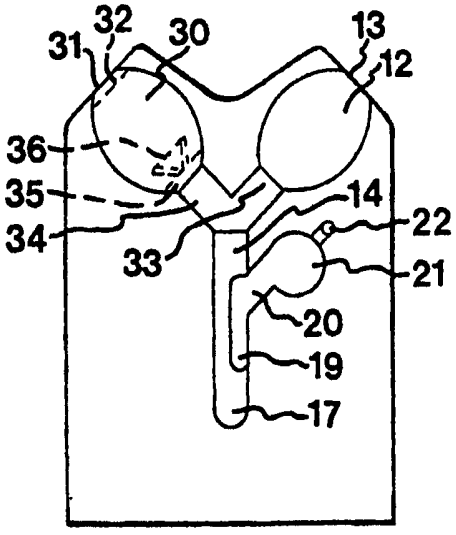


FIG.7



CUVETTE

The present invention relates to a cuvette for taking up at least one fluid and mixing the fluid with a reagent for analysing the mixture, the cuvette having at least one first cavity in which the fluid can be taken up through an inlet.

A cuvette of this type, which is used for direct optical analysis of the mixture, is previously known from U.S. Pat. No. 4,088,448. The cuvette according to this patent consists of a body member having two flat surfaces forming an optical path and spaced a predetermined distance from each other for determining the length of the optical path, and defining a cavity having an inlet by means of which the cavity communicates with the ambient atmosphere. The cavity has a predetermined fixed volume, and the predetermined distance between said surfaces enables the cavity to take up a sample by capillary action. Further, a reagent is applied to the surfaces of the cavity.

This known cuvette offers many advantages over other prior art apparatuses of the same type. By means of the cuvette, a fluid can be taken up, mixed and chemically reacted with a suitable reagent, e.g. for colour development in the same cavity as is used for the subsequent measuring operation. Thus, the cuvette according to U.S. Pat. No. 4,088,448 simplifies the sampling procedure, reduces the amount of accessory equipment and in most cases—depending on the type of analysis—considerably increases the accuracy of the analysis by making the analysis procedure independent of the skill of those carrying out the analysis.

The cuvette according to U.S. Pat. No. 4,654,197 increases the number of reactions possible in a cuvette system, by using a semipermeable membrane as a functional part of the cuvette.

The object of the present invention is to further improve these known cuvettes and to that end, the new cuvette is characterized in that it has, in addition to said first cavity, at least one second cavity adapted to take up fluid from the first cavity by capillary action without any external influence via a first channel having means for admitting fluid therein by external influence only, preferably by the exertion of centrifugal force, and that at least the second cavity contains a reagent or a fluid-modifying agent.

Thus, the cuvette according to the invention has at least two cavities defined by surrounding walls, viz. a first cavity or inlet cavity in which the fluid is taken up, preferably by capillary action through the inlet, and a second cavity in which the fluid can be taken up after centrifugation of the cuvette. Preferably, a reception cavity is provided which communicates with the first cavity through said channel. The reception cavity can be said to be divided into two sections, viz. a first, lower section for receiving heavy material taken up in the fluid, and a second, upper section forming the second cavity and serving as measuring cavity. Instead of relying on centrifugal force for fluid transport through the channel, it is also possible to exert a pressure on the fluid in the first cavity, which however presupposes a venting device. The walls of the cavities, the reception cavity and the channel, or a desired portion thereof, may be coated with reagent or the like, and an analysis can be carried out on fluid in both the first cavity and the second or the capillary section of the reception cavity,

and also in the heavier-material section of the reception cavity.

From e.g. U.S. Pat. No. 4,462,964 and U.S. Pat. No. 4,714,590 it is previously known, in an analysis cuvette, to provide capillary orifices in the fluid path. As opposed to the arrangement according to the invention, these orifices however serve to prevent fluid transport until the cuvette is subjected to centrifugation. During centrifugation, the fluid is pressed through the capillary orifices into the analysis cells. The special means which in the cuvette according to the invention prevents fluid from entering the channel might be in the form of capillary orifices as in the known devices, but such orifices would probably not be more effective than the hydrophobic filter material used. The capillary device provided between the channel of the cuvette according to the invention and its second cavity performs its function without any external influence.

One advantage of the improved cuvette according to the invention is that it can be used for whole blood sampling even if the analysis must be performed on plasma or serum. Thus, the cuvette can be used for analyses within a much broader range than the cuvettes according to U.S. Pat. No. 4,088,448 and U.S. Pat. No. 4,654,197. Another major advantage over prior art cuvettes is that the use of the centrifugal force makes it possible to carry out different reactions in different cavities, thus allowing a period of incubation before the next reagent is used. Yet another advantage is that such material as is produced or used in a reaction, such as precipitated proteins or immunoaggregates, which might otherwise interfere with subsequent reactions or measurements, can be separated by centrifugation.

The cuvette can be manufactured from glass or polymeric material. It is also possible to manufacture it from many other materials, e.g. different types of semipermeable materials, like the cuvette according to U.S. Pat. No. 4,654,197, or optically transparent or non-transparent materials. The reagent, which is provided in at least one cavity, can be deposited by evaporation, freeze-drying, spraying, screen-printing or by other techniques.

The functional parts of the cuvette may vary depending on the fluid to be analysed and the type of analysis. If the inlet cavity should take up the fluid by capillary action, the distance between the cuvette walls must be less than 1 mm, and preferably 0.7 mm. If this is not the case, the capillary action must be brought about by other means than the walls, and the wall material must be wettable with the fluid or treated to be so. The volume of the inlet cavity depends on the need of fluid in the succeeding cavities and the amount of material to be separated by centrifugation. The channel connecting the first cavity to the second or the reception cavity has low capillary action, i.e. the distance between the walls exceeds 0.7 mm. The walls defining the channel may suitably be manufactured from non-wettable material or treated so as to be non-wettable. The channel may also contain non-wettable filtering material or other means for preventing spontaneous transport of fluid from the first cavity. Thanks to this arrangement, the amount of fluid taken up becomes fairly exact and can be determined by the manufacturing process. By a suitable design of the channel, it can also be used for mixing the fluid passing through it during the centrifugation and, as indicated above, may also be provided with a reagent.

Of the two reception cavity sections, the lower section has, as stated above, low capillary action between

the walls, whereas the upper section has high capillary action. The upper section merges into the lower via a portion which can be referred to as a "wick". The "wick" may consist of capillary channels in the cuvette walls, but may also consist of a traditionally operating wick of a special design. Fluid is thus drawn from the lower section into the upper by capillary action as soon as the centrifugal force ceases acting.

The invention will be described in more detail hereinbelow with reference to the accompanying drawings schematically illustrating some embodiments.

FIG. 1 is front view of a basic embodiment of the invention,

FIG. 2 is a longitudinal section of this embodiment, and

FIGS. 3–8 are front views of other embodiments of the invention having different numbers of cavities and reception cavities of modified designs.

The cuvette in FIGS. 1 and 2 has a first wall 10 of glass or polymeric material and a second wall 11, also of glass or polymeric material. The walls 10 and 11 may also comprise several other materials, such as optical windows, semipermeable membranes, electrode material or other technical means. The walls 10, 11 define a plurality of cavities of different depths. A first cavity 12, or inlet cavity is adapted to take up a liquid sample and has such a depth that it can be filled by capillary action through a capillary inlet 13 communicating with the ambient atmosphere. However, it is also conceivable to fill this cavity by injecting the liquid sample, although one of the advantages of the invention will then be lost. The first cavity 12 may be provided with a reagent, that is an agent for reacting with the liquid sample drawn into the cavity. The reagent may be deposited on the walls of the cavity by evaporation, freeze-drying, spraying, screen-printing or in any other suitable way, depending on how the cuvette is manufactured. The first cavity 12 may also contain an agent otherwise modifying the sample. The first cavity 12 passes into a channel 14 which owing to its depth, as shown in FIG. 2, exerts low capillary action on the liquid received in the inlet cavity and has walls of hydrophobic material or walls treated with such a material. Further, the channel may also be provided with a hydrophobic filtering material, as shown at 15. These measures can also be combined. Further, the channel 14 may include a reagent or a modifying agent. The channel 14 opens into a reception cavity 16, 17 divided into two sections, viz. an upper section 16, which may also be referred to as "second cavity", and a lower section 17. The upper section or second cavity 16 exerts capillary action because of the small distance between the walls, as shown in FIG. 2, whereas the lower section 17, like the channel 14, does not exert any capillary action because of its greater depth. The walls of the lower section may be treated in the same way as the walls of the channel. Between the upper section or second cavity 16 and the lower section 17, there is provided a wick 18 connected to the upper section, but terminating at a certain distance from the bottom of the lower section. This "wick" 18 may be a conventional wick of any suitable material, but may also consist of special capillary slots in the cuvette walls or formations thereon.

When using the cuvette according to FIGS. 1 and 2, the first cavity 12 is filled with a liquid sample which in the illustrated embodiment is drawn into the cavity by capillary action through the inlet 13. The liquid sample mixes with reagent or the like provided in the cavity 12, and the mixture can then be analysed, e.g. in a photometer. If the cuvette is thereafter subjected to centrifugal force, the liquid sample or a portion thereof present in the cavity 12 can be caused to pass through the channel 14 and, during centrifugation, reach the lower section 17 of the reception cavity. When centrifugation thereafter ceases, a portion of the liquid sample will be drawn up into the upper capillary section 16 by means of the wick 18. Since the wick 18 does not reach as far as the bottom of the lower section 17, heavier material will remain therein, thus allowing separation of material. The volumes of the different cavities or sections must be so related to each other and to the volume of heavier material taken up or produced in the liquid sample, that no part of the cuvette will be excessively filled or receive an insufficient amount of fluid. Depending on the analysis to be made, neither, one or both of the sections 16, 17 can be provided with a reagent or a modifying agent. An analysis can then be made on the liquid in the upper section 16 and also on the heavier material in the lower section 17. Examples of heavier material are blood cells collected in the section 17 when analysing a blood sample.

FIG. 3 shows an embodiment of the invention which is more useful in practical application. The cuvette may be designed in the same way as in FIGS. 1 and 2 and has a first cavity 12 with an inlet 13, a channel 14 with hydrophobic obstacles and a reception cavity 17. However, the upper section or second cavity, here designated 21, of the reception cavity is offset with respect to a centre line passing through the first cavity and the lower section of the reception cavity 17. The second cavity 21 communicates with the reception cavity 17 by a capillary channel 20 making an angle with the centre line passing through the first cavity 12 and the reception cavity 17. A capillary formation or wick 19 of the same type as the wick 18 is connected with one end to the Capillary channel 20 and extends a certain distance downwards towards the bottom of the section 17, but terminates at a safe distance therefrom for the same reason as in the previous embodiment. The second cavity 21 is here connected to a venting device in the form of a channel 22 opening into the ambient atmosphere for preventing the formation of air inclusions. In this cuvette, the measuring or reaction cavity 21 is thus not located in the fluid path existing during the centrifugation of the cuvette and may thus be provided with a reagent incompatible with the heavier material in the liquid. This simple cuvette solves a number of analysing problems. Reagents or other agents can be deposited in several places by different techniques. Incubations over suitable times are possible in the first cavity 12 and in the reception cavity 17 during centrifugation and, of course, in the second cavity 21. If several reagents or the like are required on different occasions after a separation process, the cuvette must have more than three cavities, where a second cavity serves as an inlet cavity for a new cycle of centrifugation, as will appear from the following description.

The cuvette in FIG. 4 thus has a second reception cavity 28 communicating with the second cavity 21 through a channel 27 which, like the channel 14, is provided for Preventing spontaneous liquid transport by capillary action. The second reception cavity 28 r-an be used as a further measuring cavity and may be provided with a reagent or the like. Liquid present in the second cavity 21 can be caused by centrifugation to pass through the channel 27 to be taken up in the reception

cavity 28. After a predetermined time and optionally after mixing with a reagent, the liquid can be subjected to analysis in the cavity 28. One of the advantages of this embodiment of the invention is that a reagent can be provided in the cavity 21 and the liquid received there passed, after a predetermined time of incubation, to the reception cavity 28 after centrifugation of short duration, and the liquid is then mixed in the cavity 28 with a new reagent or the like in order to be analysed after a predetermined time of incubation.

FIG. 5 shows an embodiment which, in addition to the first cavity 12, the channel 14 and the reception cavity 17, has a second cavity 21, a third cavity 21' and a fourth cavity 21" as well as a second channel 14' and a third channel 14", a second reception cavity 17' and a third reception cavity 17" as well as a first capillary channel 20, a second capillary channel 20' and a third capillary channel 20". A liquid taken up in the first cavity 12 is passed, as described above, into the reception cavity 17 by centrifugation, from where it is taken up in the second cavity 21 by capillary action through the wick 19 and the capillary channel 20. From the second cavity 21, the liquid is transported to the reception cavity 17' via the channel 14', also by centrifugation, to be drawn from there up into the third cavity 21' by means of a wick 19' in the same manner as in the preceding step. Similarly, the liquid is taken up in the fourth cavity 21" via the reception cavity 17", the wick 19" and the channel 20". There are not very many analyses having such a complicated reaction pattern as to necessitate a cuvette of the embodiment now described. However, this embodiment clearly shows the versatility of the invention. In the last-mentioned embodiment, the venting channel 22 is connected to the last cavity 21" in the series of cavities.

FIG. 6 shows a further embodiment which is a combination of the embodiments of FIGS. 3 and 4. Thus, to a reception cavity 17 are connected two channels 20, 24 which are each connected to a second cavity 21, 25 and each have a wick 19, 23. The cavities 21, 25 each have a venting channel 22 and 26, respectively. The embodiment in FIG. 6 can be used for performing two analyses which must be carried out after different times of incubation. Since two analyses can be performed after a single centrifugation, the cuvette according to FIG. 6 can be time-saving in many cases.

One practical example of the versatility of the invention is the analysis of urea and alkaline phosphatase from whole blood in the cuvette according to FIG. 6. The cuvette wall 10 with the recesses defining the cavities can be manufactured from cellulose-based resin while the other wall, forming a lid, can be cut from a sheet of the same material.

The surfaces of the cavities depending on capillary force can be treated by corona discharge or in any other way for increasing wettability. The hydrophobic channels 14, 14', 14" and 27 can be treated with silicone fluid, and a filter consisting of a small piece of sintered polypropylene can be pressed into place in the upper part of these channels. A mixture of glycine, magnesium chloride, paranitrophenyl phosphate and a carrier agent, giving a pH of 10.5 when dissolved in plasma, is printed on one or both of the large surfaces defining the second cavity 21. On the surfaces defining the cavity 28 in FIG. 6 is printed a mixture of sodium hydroxide and a carrier agent. To one of the walls defining the cavity 25 is applied a mixture of urease and an alkaline buffer, and on the corresponding area of the opposite wall is applied a substantially transparent material of cellulose ester containing a pH indicator with an indicator range within the acid area. The first cavity 12 and the reception cavity 17 may contain heparin to prevent coagulation If the reaction time is long. The two walls which according to FIG. 2 form the cuvette can be joined together by welding or gluing. Both methods give excellent results.

In the use of a cuvette according to FIG. 6, which has been treated in the manner just described, the cuvette is contacted with a whole blood sample and placed in a special centrifuge photometer. Centrifugation is started, and the blood is passed into the reception cavity 17. After 60–90 seconds, the blood cells have been separated, and the centrifuge is stopped. Plasma is now drawn up into the cavities 21 and 25 through the channels 20 and 24. The photometer may have an initial measurement as reference, otherwise analysing starts by monitoring the kinetic turnover or reversal of the pH indicator because of the ammonia produced by the urease action on the sample urea in the cavity 25. As the urea value is read, the alkaline phosphatase reaction proceeds in the second cavity 21 and after a predetermined time, the centrifuge is started in order, after a short time, to bring the reaction to a stop when the liquid has been contacted with the sodium hydroxide in the cavity 28, which also develops a yellow colour of digested substrate. After measuring the colour in the cavity 28, the data received is processed and the analytical values are presented.

It may sometimes be desirable to dilute or wash the drawn-up fluid with a liquid which should be applicable in one or more cavities provided therefor. To this end, a cuvette of the design shown in FIG. 7 can be used. Here, the cavity 12 is connected in parallel with a cavity 30 for taking up said liquid. The two cavities 12 and 30 each have an outlet channel 33 and 34, respectively, both of which open in the channel 14. During centrifugation, fluid and liquid in the cavities 12 and 30, respectively, will flow into the channel 14 and through this channel into the reception cavity 17 and so forth, as in the preceding embodiments.

The diluting or washing liquid can be sucked into the cavity 30 in connection with the analysis, but it can also be supplied in advance, suitably when applying the reagent, in which case the liquid must be sealingly enclosed, which can be done by means of sealing plugs or membranes provided in the inlet and the outlet of the cavity. It is also conceivable to place a capsule of suitable material in the cavity 30. When the cuvette is to be used, the two seals can be penetrated by means of a suitable tool. It is also possible, as illustrated at 36, to provide some type of perforation means 36 in the cavity. When the cuvette is subjected to centrifugation, the perforation means 36 will thus be urged into engagement with the seal 35 in the outlet so as to penetrate it.

It is also conceivable to connect the cavity with washing or diluting liquid in series with the fluid reception cavity 12. This can be done, for instance, by modifying the cuvette according to FIG. 5 in the way shown in FIG. 8. The cavity, which in the embodiment according to FIG. 5, serves as second cavity 21 is here used as first cavity 12 by being provided with an inlet 39. The first cavity in FIG. 5 here forms a cavity 38 for receiving diluting or washing liquid which, like the fluid in the embodiments described above, is supplied by means of a channel 41, a reception cavity 40 and a liquid-drawing capillary formation 42, to the fluid reception cavity 12

and transported, if so desired, to the succeeding cavities in the same manner as in the embodiment of FIG. 5. The diluting or washing liquid can be drawn into the cavity 38 by capillary action in connection with the analysis, but many times it is instead more conveniently applied in advance and sealingly enclosed in the cavity in the same manner as in the cavity 30 in FIG. 7.

In certain analyses, it may be desirable to retain part of the fluid or the diluting or washing liquid which by the centrifugation has reached the respective reception cavity 17 and 40, in this cavity. Suitably, the cavity is widened, as shown at 37, so as to have a volume exceeding the volume of the cavity 21 and 12, respectively. After a second centrifugation, in which e.g. the cavity 21 has been emptied, fluid is therefore again drawn up from the cavity 17.

The drawings show all the cavities as defined by sealing walls, but it is evident that one or some of these walls can be replaced by a semipermeable membrane, as stated in U.S. Pat. No. 4,654,197.

The invention will be further illustrated by Examples 1 and 2, relating to the determination of hemoglobin and glucose in whole blood, and glucose and protein in serum or plasma, respectively, using the cuvette described above.

EXAMPLE 1

Determination of Hemoglobin and Glucose in Whole Blood

The red cells of the blood, the erythrocytes, carry inside their semipermeable membrane, primarily of lipides and proteins, a plurality of water-soluble chemical substances of both low- and high-molecular type. An example of the high-molecular type is the oxygen-transporting protein hemoglobin and an example of the low-molecular type Is glucose which is a necessary energy substance for sustaining metabolism. Low-molecular substances often exist both intra- and extracellularly, while high-molecular substances often cannot pass through the membrane of the erythrocytes. When determining hemoglobin or glucose in whole blood, the membrane of the erythrocytes is ruptured, e.g. by a detergent or an osmotic shock or a combination thereof, and the substances contained in the erythrocytes become available for chemical analysis.

Hemoglobin

In a cuvette according to the invention, e.g. FIG. 3, the cavity 12 is supplied with a dry chemical reagent consisting of

0.30 mg sodium deoxycholate
0.15 mg sodium azide
0.15 mg sodium nitrite
0.1 mg non-reactive ingredients

The reagent composition for a certain cuvette quantity is dissolved in a small amount of water and Pluronic P85 ®. The reagent composition has such a viscous consistency that it can be uniformly applied over the surface in the cavity 12, e.g. by screen-printing or dabber printing. The reagent Composition used produces, together with hemoglobin, a hemoglobin azide complex which can be determined photometrically in the cavity 21. The cuvette with hemoglobin reagent is used such that the cavity 12 is supplied with whole blood. The reagent dissolves into the blood, and the chemical reaction forming a hemoglobin azide complex is finished after about 45 seconds. The contents in the cavity 12 are transferred, e.g. by centrifugal force, into the cavity 21 where a clear low-turbid solution can be analysed by photometry. The distance between the walls in the cavity 21 is about 0.13 mm.

Glucose

1 kU GDH, glucose dehydrogenase
220 U NAD
0.3 mmol MTT
250 g White Saponin ®
50 mg Pluronic P85 ®
250 µl water subjected to ion-exchange

The components included are finely divided into a suspension which is suitable to be used for coating surfaces by different printing techniques, such as silk screen printing, cylinder printing etc. This type of suspension is suitable for coating cuvettes according to the invention. In certain cases, surface-tension reducing substances may be added for facilitating the coating of hydrophobic plastic materials. In order to adapt the suspension to different coating equipment, the viscosity can be varied by adding suitable high-molecular polymers. The choice of high-molecular polymers is not critical, but affects the dissolving rate of the dry reagent. Among usable polymers may be mentioned polyethylene glycol, polyvinyl pyrrolidone, dextran and different cellulose derivatives. The choice of polymer can also be made with a view to stabilising the suspension. On the basis of known preparation techniques in e.g. the foodstuffs or cosmetics industry, the reagent can be adapted to different surfaces.

The reagent for glucose in whole blood is placed, as described above, in a cuvette according to the invention of the type shown in FIG. 3. The glucose reagent is placed in the cavity 12. The transfer of reagent into the cavity 21 can be achieved, e.g. by centrifugal action. The cavity 12 is filled with whole blood, and the glucose reagent brings about a conversion of glucose into a photometrically measurable colour at end-point after about 3 minutes. The transfer into the cavity 21 can be effected after the red blood cells, the erythrocytes, have been ruptured, i.e. about 1 minute after. In the same way as in the case of hemoglobin, photometering is carried out in a low-turbid clear aqueous solution. The distance between the walls in the cavity 21 is about 0.14 mm for glucose determination in whole blood. The photometric method for determining glucose and hemoglobin in whole blood is advantageously performed by a two-wavelength measurement.

EXAMPLE 2

Determination of Glucose and Protein in Serum or Plasma

When determining an analyte in plasma or serum, the red blood cells, the erythrocytes, should be excluded. A cuvette according to the invention is especially well suited for analysing in plasma or serum when the cuvette has several cavities and the communication between the different cavities is maintained by capillary force and centrifugal force. Blood is drawn into a cavity, often by capillary force by direct sampling, and plasma or serum is transferred, after centrifugation of the cuvette, by capillary force into a cavity containing a reagent composition, specifically suited for determining the analyte.

Glucose in Plasma or Serum

Reagent composition, 1 ml:

1 kU GDH, glucose dehydrogenase enzyme
220 U NAD
0.3 mmol MTT
50 mg Pluronic P85 ®
250 μl water subjected to ion-exchange

The reagent chemicals included are treated as in the previous Example for determining glucose in whole blood. Any modification of the reagent composition to achieve an adequate function, such as dry reagent, and adhesion to the walls of the cuvette cavity complies with the description in the previous Example.

For determining glucose in plasma or serum in a cuvette according to the invention, the cuvette according to FIG. 3 is advantageously used. The reagent composition described above is applied in the cavity 21, e.g. by printing technique, uniformly over the surface thereof. After drying, the reagent passes into what is often referred to as dry reagent. A lid is placed over cavities and other channels in the structure. Whole blood is sampled and flows into the cavity 12, e.g. by capillary action. After sampling, the cuvette is centrifuged, and after completed centrifugation the cavity 21 is filled with plasma or serum by capillary action. The red blood cells have been removed by centrifugation and cannot fill the cavity 22. A-he reagent composition dissolves in serum or plasma, and the chemical reaction permits a specific determination of glucose. The chemical reaction, i.e. the glucose content, can be read directly in the cuvette by photometric technique.

Protein in Serum or Plasma

Reagent composition:
1 mmol lithium tartrate
1 mmol copper tartrate
7 mmol lithium hydroxide

These chemical substances are dissolved in a suitable amount of water. In order that the solution should be given the correct viscosity for application in a cavity by printing technique, the solution is evaporated. The application of the reagent by printing technique is facilitated if the dry reagent additionally contains about 0.5-2% lithium lauryl sulphate and about 1-5% polyvinyl pyrrolidone/polyvinyl acetate copolymer and optionally a plasticiser.

The reagent is applied in the cavity 21 in a cuvette according to FIG. 3. The cuvette functions in the same manner as the cuvette used for glucose determination in plasma or serum.

The cuvette according to the invention can be used for many types of analyses and is especially well suited for routine-type blood analyses, such as determination of glucose, urea-nitrogen in blood, albumin, bilirubin, total protein etc., particularly on the basis of whole blood, and for a large number of other analyses. Thus, the invention must not be considered restricted to what has been described above, but may be modified in several different ways within the scope of the accompanying claims.

We claim:

1. A cuvette for taking up at least one fluid and for mixing a fluid with a dry reagent for analyzing a mixture, wherein said cuvette comprises:

a) at least one capillary first cavity having an inlet and constructed and arranged to take up a fluid by capillary action alone;

b) a first channel having a non-capillary and non-spontaneous fluid transporting function operative only under external influence by application of a centrifugal force on the cuvette;

c) a centrifugation reception cavity communicating with said at least one capillary first cavity via said first channel and constructed and arranged to exert no capillary action:

d) at least one capillary second cavity constructed and arranged to take up fluid by capillary force alone; and

e) a first capillary transporting means projecting into said centrifugation reception cavity, being connected to said at least one capillary second cavity and constructed and arranged to transport fluid by capillary action from said centrifugal reception cavity into said at least one capillary second cavity.

2. A cuvette as claimed in claim 1, further comprising a capillary channel provided between said reception cavity and said at least one capillary second cavity, an end portion of said first capillary transporting means facing away from said reception cavity being fixed in said capillary channel.

3. A cuvette as claimed in claim 1, further comprising a second reception cavity communicating with said at least one capillary second cavity via a second channel corresponding to said first channel.

4. A cuvette as claimed in claim 3, wherein said second reception cavity has a second capillary transporting means corresponding to said first capillary transporting means and connected to a second capillary channel opening in a capillary third cavity.

5. A cuvette as claimed in claim 4, further comprising at least a third reception cavity, a third channel, a third capillary transporting means, a third capillary channel and a capillary fourth cavity connected to said at least one capillary second cavity via said capillary third cavity.

6. A cuvette as claimed in claim 5, having a plurality of channels and reception cavities, and capillary transporting mans, capillary channels and cavities, wherein all channels and reception cavities extend along parallel lines making an angle with parallel lines along which the capillary channels extend.

7. A cuvette as claimed in claim 1, wherein at least two capillary cavities are connected to each reception cavity.

8. A cuvette as claimed in claim 1, wherein said capillary transporting means consists of a wick.

9. A cuvette as claimed in claim 1, wherein all cavities and/or reception cavities are coated with reagents or fluid-modifying agents.

10. A cuvette as claimed in claim 1, wherein at least one cavity for receiving diluting or washing liquid is connected in parallel with said at least one capillary first cavity, said two cavities having outlets connected to said first channel.

11. A cuvette as claimed in claim 1, wherein at least one cavity for receiving diluting or washing liquid is connected in series with said at least one capillary first cavity via a channel, a reception cavity and a capillary transporting means.

12. A cuvette as claimed in claim 10, wherein said cavity for receiving diluting and washing liquid is provided, at an inlet and an outlet thereof, with means for sealingly enclosing a liquid, the means for sealingly enclosing the liquid provided in said outlet being rupturable by a penetrating means disposed in the cavity and activatable by centrifugal force.

13. A cuvette as claimed in claim 1, wherein at least one reception cavity has a larger volume than the succeeding cavities which are arranged to take up fluid from said reception cavity.

14. A cuvette as claimed in claim 1, wherein at least one of the cavities is covered with a hydrophillic or hydrophobic semipermeable membrane containing a reagent.

15. A cuvette as claimed in claim 1 in which said capillary second cavity is offwet with respect to said first channel and wherein said capillary second cavity communicates with the ambient atmosphere through a vent.

16. A cuvette as claimed in claim 1 in which said capillary second cavity is in line with said first channel and between said first capillary cavity and said centrifugation reception cavity.

17. A cuvette as claimed in claim 1 wherein said capillary second cavity contains a dry reagent or a fluid-modifying agent.

* * * * *

Date August 30, 1993

To [illegible]

From [illegible]

This is in response to the question raised by the Examiner at the Food and Drug Administration concerning the use of the HemoCue® Photometer and Microcuvettes for certain unusual hemoglobin types.

First, as we understand it, the Examiner has asked whether the HemoCue®-system can analyze samples containing such as [illegible] or abnormal hemoglobines as in thalassemia. The HemoCue® Microcuvette contains 4 % desoxycholate, which is added specifically to provide a complete lysing of red cells of all types including those mentioned above. As a matter of interest, this agent is able to lyse even bacterial cell-walls. The system therefore provides complete lysing of all types of red cells.

Additionally it was asked how the HemoCue®-system would perform, if such abnormal cells were not analyzed immediately. The basis of this question lies in the process used by the instrument to make its determination. While it is true that the instrument follows the progress of lysing, searching for a time when the reaction is complete, then making its measurement, the instrument is able to make an accurate determination even for delays up to 20 minutes. In such instances, where the sample is put aside for a few minutes prior to analysis, allowing the sample to reach lysed steady-state, the instrument takes three readings and provides the mean as its answer. In this situation, the three measurements would be virtually

AKTIEBOLAGET LEO
DIAGNOSTICS
Box 941
S-251 09 Helsingborg
Sweden
Telephone 042-10 40 00
Telex 72533 leoswed s

For technical assistance contact
Sven-Erik Nilsson

Date of latest labeling revision:

HemoCue®

A Microcuvette for The Determination of Total Hemoglobin in Blood

Figure 1 - 5 - How to use the HemoCue® Microcuvettes

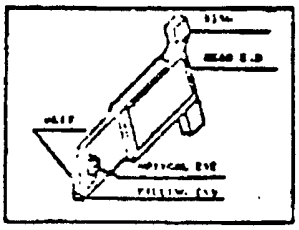


Figure 1

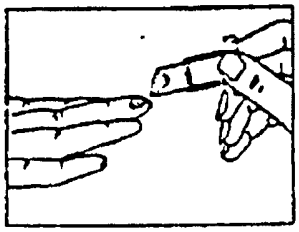


Figure 2

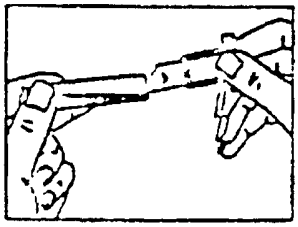


Figure 3

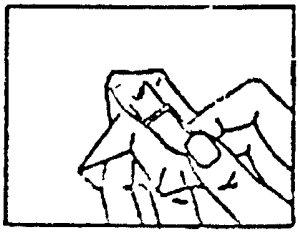


Figure 4

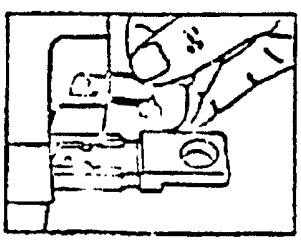


Figure 5

16 STABILITY OF FINAL REACTION

Measurement of a HemoCue® Microcuvette should be made as soon as possible after the blood has been drawn into the cuvette. If the microcuvettes are stored on a sturdy table in a flat position they will give clinically meaningful results for up to 20 minutes.

Test of "Stability of Final Reaction"

In a study to confirm that a filled HemoCue® Microcuvette will give clinically meaningful results for at least 20 minutes 8 different specimens of EDTA-blood were used. The analytical range was approximately 55 to 202 g/l.

The following procedure was used:

1 Fill a HemoCue® Microcuvette with blood.

2 Place the microcuvette in the cuvetteholder of the HemoCue® Photometer and push the cuvetteholder to its measuring position.

3 Note the result when it is shown in the digit display.

4 Pull the cuvetteholder to its outer position and remove the microcuvette. Let the microcuvette rest in a flat position on a sturdy table.

5 Wait one minute and repeat steps 2 - 4 until 31 measurements have been performed.

6 Repeat steps 1 - 6 with all the eight EDTA-blood specimens.

AKTIEBOLAGET LEO
DIAGNOSTICS
Box 541
S-251 09 Helsingborg
Sweden
Telephone 042-10 40 00
Telex 72133 leoswed s

For technical assistance contact
Sven-Erik Nilsson

HemoCue® is a registered trade mark of Aktiebolaget Leo

Date of latest labeling revision:

HemoCue®

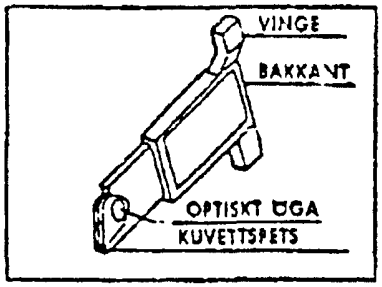
A Microcuvette for The Determination of Total Hemoglobin in Whole Blood

3.5 Utförande av hemoglobinbestämning

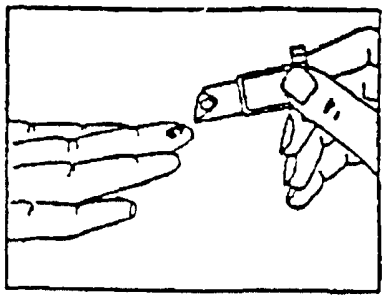
1 Ställ fotometerns strömbrytare i läge "ON". I avläsningsfönstret ses bokstäverna "Hb". Drag ut kuvettsläden till sitt yttre läge. När "Hb" i avläsningsfönstret ersatts av tre blinkande streck, är fotometern klar för mätning.

2 Tag ut erforderligt antal kuvetter ur burken och sätt omedelbart på locket. OBS! Avlägsna ej torkmedlet. Utför kapillärprovtagning på vedertaget sätt (första bloddroppen avtorkas), resp. tag fram det venblod som skall analyseras.

3 Fyllning av kuvetten

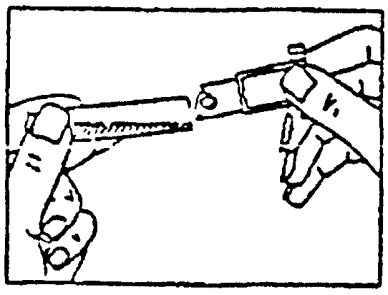


Håll kuvetten i den bakre vingförsedda delen.



Kapillärblod

För kuvettspetsen mot bloddroppen. Se till att bloddroppen är tillräckligt stor för att fylla hela kuvetten *på en gång*.
Undvik blod på kuvettens utsida.



Venblod (väl blandat!)

Luta röret med venblod och för kuvettspetsen mot blodytan så att hela kuvettens fylls *på en gång*.
Undvik att få blod på kuvettens utsida.
Kuvetten skall vid provtagningen fyllas helt. Inga luftblåsor får finnas i det optiska ögat. Mindre luftblåsor längs kanten påverkar ej resultatet.

Int. Cls.: 9 and 10

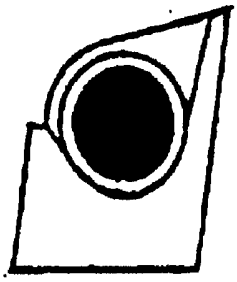
Prior U.S. Cls.: 21, 23, 26, 36, 38, 39 and 44

United States Patent and Trademark Office

Reg. No. 2,629,645

Registered Oct. 8, 2002

TRADEMARK
PRINCIPAL REGISTER



HEMOCUE AB (SWEDEN CORPORATION)
KUVETTGATAN 1
SE-262 23 ANGELHOLM, SWEDEN

FOR: COMPUTERS; ELECTRONIC APPARATUS FOR LABORATORY ANALYSIS, NAMELY, SPECTROPHOTOMETERS; AND SCIENTIFIC AND MEASURING APPARATUS AND INSTRUMENTS, NAMELY, PHOTOMETERS, LABORATORY GLASSWARE, BEAKERS, FLASKS, ELECTRONIC SENSORS FOR MEASURING LIQUIDS, BAR CODE READERS AND QUALITY CONTROL MATERIALS FOR CALIBRATION PURPOSES, NAMELY, CALIBRATORS FOR USE IN CALIBRATING AND VERIFYING THE ACCURACY OF PHOTOMETERS AND ELECTRONIC SENSORS USED FOR MEASURING LIQUIDS, IN CLASS 9 (U.S. CLS. 21, 23, 26, 36 AND 38).

FIRST USE 12-17-1982; IN COMMERCE 9-0-1988.

FOR: MEDICAL APPARATUS AND INSTRUMENTS FOR MEDICAL ANALYSIS, NAMELY, CUVETTES AND MICROCUVETTES , IN CLASS 10 (U.S. CLS. 26, 39 AND 44).

FIRST USE 12-17-1982; IN COMMERCE 9-0-1988.

PRIORITY CLAIMED UNDER SEC. 44(D) ON FED REP GERMANY APPLICATION NO. 39875026.2, FILED 12-30-1998, REG. NO. 39875026, DATED 4-1-1999, EXPIRES 12-31-2008.

SER. NO. 75-676,649, FILED 4-7-1999.

MARY CRAWFORD, EXAMINING ATTORNEY